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RESEARCHES ON TETANUS*

VI. The Production of Reflex Motor Tetanus by Intraspinal Injections of Tetanus Toxin

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Submitted for publication September 17, 1937

Thirty-five years ago Meyer and Ransom (1) reported a series of experiments on three cats and one rabbit in which large and rapidly fatal doses of tetanus toxin were injected into the substance of the lumbar segment of the non-transected spinal cord. The volume of the injections varied from 0.02 to 0.1 cc. The three cats and the rabbit that were used lived from 10 to less than 24 hours. The outcome of these toxicological experiments was unexpected, *for within 2 hours after the recovery from the ether anesthesia* symptoms of that frightful complication, tetanus dolorosus, were so fully developed that the slightest touch on a hind foot or on the tail caused the animal to cry out and to bite at the part to which the pain was attributed. Next to the tetanus dolorosus the most significant result of these experiments was the appearance in so short a time as three hours of localized rigidity of the hind limbs of the cats, while the muscles of the rabbit remained unaffected. In addition, the cats showed only some degree of reflex excitability.

Thirteen years later Meyer and Fröhlich (2) transected the cord of a rabbit (weight 3.6 k.) at the level of the first lumbar vertebra, and injected $\frac{1}{15}$ to $\frac{1}{18}$ of a lethal dose of tetanus toxin into the distal segment. Twenty-four hours later a reflex motor tetanus made its appearance in both hind limbs. This reflex tetanus was characterized by the appearance of repeated jerks of the hind limbs—a type of

* A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of investigations on tetanus.

response referred to as "Streckstösse" or "Jaktationstetanus." These repeated jerks gave place the next day to extreme rigidity of the hind limbs. In another experiment the spinal cord of a kitten weighing 1000 grams was divided at the level of the thirteenth thoracic vertebra and $\frac{1}{5}$ of a lethal dose of toxin injected distally. Eighteen hours later there was increased reflex excitability and the animal cried out when seized; otherwise it was normal. On the fourth day the absence of trismus and of general symptoms of tetanus was recorded, but both hind limbs exhibited local tetanus to such a degree that it persisted during deep chloroform anesthesia.

The above experiments, with the intact and the severed cord, have been adduced to prove that both of the cardinal symptoms of natural tetanus, namely, muscular rigidity and reflexly induced spasms, are solely of central origin. In an endeavor to learn whether the local rigidity produced by tetanus toxin is due to the direct action of the toxin on neuromuscular terminals, or to its action on the motor cells of the cord, we have carried out the series of spinal cord injections reported in this paper. We believe these experiments show that it is possible to produce tactile reflex motor tetanus by spinal cord injections without inducing the slightest sign of muscular rigidity (Starre), or of that frightful complication, tetanus dolorosus. In eliminating the complications that vitiated many of our first experiments in 1935, as they also did those of our predecessors, we have succeeded in producing a physiological experiment by means of which one action of tetanus toxin on central neurons is clearly demonstrable.

The following protocol of one of our experiments will serve as an example of the successful experiments carried out in 1935.

Protocol: Dog A 21

December 2, 1935. White and black hound, male, about a year old, weight 7.3 kgm.

Ether anesthesia.

The operative field was shaved and prepared with iodine-alcohol technique. The dorsal spine and laminae of the third lumbar vertebra were removed. The dura was not incised, but was displaced medially so as to permit the introduction of a 25-gauge needle on the right side. The needle entered the spinal cord laterally just below the midline and its point was aimed at the region of the right anterior horn. The needle was attached to a capillary pipette which was calibrated to one-thousandth of a cc. The distal end of the pipette was inserted into a

piece of thick rubber tubing, bearing a screw clamp. By turning the screw a few degrees it was possible to aspirate into or to expel from the pipette as little as one one-thousandth of a cc. The tip of each pipette was ground to fit accurately a particular needle, thus preventing leakage at the junction.

After the needle had entered what was thought to be the region of the motor horn the screw clamp was gently tightened and 0.007 cc. of tetanus toxin in dog serum was slowly injected into it. The 0.007 cc. of injected material was made up of four parts of normal dog serum to one part of tetanus toxin, batch 24a. The lethal dose of this toxin for a dog weighing 7.3 kgm. was 0.124 cc.; therefore, the amount injected into the cord was 1/88 of a minimal lethal dose. The operation was completed at 3.30 P.M.

Progress Notes: December 3, 11.00 A.M. The animal is unusually timid but it is apparent when it walks that the right hind leg is slightly weak. 4 P.M. The weakness in the right leg is more apparent than when observed earlier in the day. The dog definitely spares his leg when walking. While standing there are slight tremors in this leg. Tendon reflexes in right hind limb are slightly hyperactive. No sign of tetanus dolorosus is present.

December 4, 11 A.M. The dog is showing definite signs of motor tetanus in the right hind limb. There are constant tremors. When the leg is touched it is jerked away quickly. This is followed by several short contractions. Touching the left hind leg or other part of the body elicits no such response. 3 P.M. The dog can stand and walk, but holds his right leg off the floor most of the time. Whenever the right foot touches the floor, it is withdrawn spasmodically. This is repeated indefinitely. In this raised position there are continual minor jerks of the leg. If the dog is placed on the left side, the spasmodic seizures are almost eliminated but rhythmic contractions of a smaller degree are still observable. In this position if one takes hold of the right foot and tries to flex the leg at the joints by continued gentle but firm pressure, the leg is thrust firmly against the fingers. If one keeps on pressing upward, the force of the thrust against the fingers is increasingly violent. There is not the slightest degree of permanent contraction of this leg and the leg muscles do not have a trace of real rigidity. When the animal is lying on the left side, the right leg does not assume a position of extension unless the foot touches the floor or wall (in which event such stimulation causes a thrust movement); in the absence of stimulation the leg is slightly flexed and drawn up close to the body. There are no cries or evidences of sharp pain such as are associated with tetanus dolorosus. The dog drinks freely but has little appetite for solid food. There are absolutely no signs of trismus or other symptoms of descending tetanus. Moreover, reflexes of the right leg can be induced only by touching the skin of that leg. Touching any part of the skin of the left leg does not induce reflex thrusts of the right leg.

December 5, 11.30 A.M. The reflex symptoms have become greatly exaggerated and now involve not only the right leg but also the tail and to a lesser degree the left hind leg. The animal is constantly moving about attempting to place itself in a comfortable position; it rolls from one side to the other and at

times attempts to struggle to its feet. At one time during the observation it managed to get onto its feet by pressing the left side of its body against the leg of a table, thus bracing itself. While standing, the right leg was extended with the right foot bent under so that the ventral surface of the paw was on the floor. This position was maintained for only a few seconds, when a spasmodic seizure of the right leg and then the left caused the dog to fall down. When the dog was placed on its left side definite responses to sensory stimuli were observed as follows: Touching the pads of the right foot causes the leg to be thrust violently against the hand. These reflex thrusts are repeated several times before a period of relaxation ensues. The thrusts are decidedly more vigorous than yesterday, and differ entirely from the steady contractures that appear in every instance of local tetanus, since the leg is freely movable at all joints. Flexion of all of the joints is easily induced as the animal attempts to withdraw the foot from the steady upward pressure of the hand.

The left hind leg also responds today to sensory stimulation but in a markedly less degree than the right leg. This is evident from the fact that the animal can lie with the left leg on the floor with only minor evidence of reflex stimulation. As was the case yesterday, the spinally induced motor tetanus cannot be called forth by touching the skin of any part of the trunk, forelegs or head. As was true yesterday, there is no sign of abnormal reflex activity except that due to localized lumbar injection of the toxin.

The condition is one of extreme reflex motor excitation. There are only brief periods of cessation of the continual or rapidly alternating spasmodic seizures; such periods occur when the animal is suspended by holding it free of the floor, with one's hands under the forelegs, or when it lies on its left side with the right leg resting on a bag and not touching the floor. The intervals between reflex spasms under these circumstances last for about 5 or 6 seconds only. When the animal is lying on its left side and is comparatively quiet the respirations are from 20 to 22 per minute. The pulse rate is 140 per minute. There is no retention of urine.

When the animal was etherized, the same results were obtained as in experiments of combined tetanus dolorosus and motor tetanus. All reflex activity was immediately abolished after the first few whiffs of ether; indeed, even while the animal was still struggling to remove its head from the ether cone. After removal of the cone, the animal slept for about 5 minutes, but long before consciousness returned the reflex excitability returned and became increasingly evident with the full return of consciousness.

There is no evidence in this animal of the toxin ascending in the cord, or diffusing into either posterior horn. The occurrence of slight reflex motor tetanus in the left hind limb is undoubtedly due to the direct deposition of a minute amount of toxin in the left anterior horn. 3.15 P.M. When the left foot is grasped, there is a more decided thrust than occurred at 11.30 A.M. This indicates that the poisoning of the left center is becoming more and more apparent, showing here also a period of incubation. Rectal temperature: 103.5°.

December 6, 4 P.M. In general the animal seems weaker today, although

when held up it takes fluids freely. Drank about a pint of milk. Today it is able to lie on either side but seems to prefer to lie on the right side, in contrast to yesterday, when it was so reactive that touching the floor caused the most violent thrusts and contractions of the right leg. When the right leg is touched or the foot grasped, the animal thrusts its leg violently against the hand. During a tetanic spasm of this nature the muscles are hard and the joints are almost rigid. But in the interval between spasms the joints can be flexed quite easily although the posture of the limb at the moment is that of extension. The left leg today is much more excitable than yesterday, and the thrusts are firmer; the joints are readily flexed as the animal withdraws and extends the leg during stimulation. Although the forelegs are held in an extended position when lying quietly, the animal bends them voluntarily when, for instance, it makes an effort to get into a more comfortable position or to get up. Touching the trunk, forelegs or head does not elicit spasms of the hind legs. In general, the picture today is not so violent as yesterday. The spasms are not so continuous; at times there are brief periods of rest. The respiratory and pulse rates are about the same as yesterday. Rectal temperature: 101.8°.

When the attendant took the animal up to its cage, the wound opened a little and there was a discharge of bloody fluid.

December 7, 12 N. Observed by Dr. Abel, who noted a marked kyphosis not present at 6 P.M. yesterday. The animal lies on its right side today with the right leg extended and the left slightly flexed. There are periods of rest of 2 or 3 minutes when there are no spasms. But when the foot pads of either hind foot are grasped or stroked, there is an immediate spasm of both hind legs, the thrust of the right leg still being stronger than that of the left. It seems, however, that a greater stimulation is necessary to bring forth a reaction than yesterday. Touching or handling the forelegs, trunk or head does not cause reflex spasms. When the left hind foot is grasped and pressed upward, the entire limb is easily folded up against the body. This is true also of the right leg except that this leg is so much more reactive to sensory stimuli that the flexed position is less easily enforced. The forelegs are entirely normal.

The animal had a bowel movement during the night. Today there is some retention of urine. Firm pressure on the abdomen expelled a fair amount of urine. While this was being done there were violent spasms of both hind legs. Respiration and pulse were about the same. The temperature was 99°.

December 8, 10.30 A.M. The animal is still alive and conscious, though it refuses fluids. Thrusts of both hind legs can still be elicited by touching the skin of the legs, but a stronger stimulation is necessary today than yesterday. The thrust of the right leg is more pronounced than that of the left. The animal now has periods lasting 4 minutes or more when there are no reflex spasms. The kyphosis is much more marked today than yesterday. There is urinary incontinence. When the temperature was taken the mercury of the thermometer could not be raised to 94°. Respirations are shallow. The pulse is rapid and weak.

December 9. The animal is dead.

Autopsy

December 9, 4 P.M. The wound was superficially infected but this infection did not extend down beneath the fascia. There was some red hepatization of the left lung and a small amount of pus in the left pleural cavity. There was a dislocation of the 3d lumbar vertebra.

This dislocation occurred sometime after 6 P.M. December 6th and noon of December 7th. A similar complication did not occur in any of our other experiments and it is safe to say the dislocation did not alter the usual course of anterior horn injections. The pneumonia could account for the fever.

Comment: With the above technique as used by us in 1935 it is obvious that the operator could not be certain as to the site of injection. In a few of the experiments there were absolutely no symptoms subsequent to the injection, and it was assumed that the needle passed through the cord, causing the toxin to be injected into the spinal fluid. That this actually occurred was confirmed by using a filtered solution containing finely divided charcoal. The other possibility accounting for negative results is that the toxin leaked out by way of the needle track. The indisputable evidence that the toxin had been placed in the substance of the anterior cord is found in the subsequent occurrence of exaggerated reflex states. The dogs in which the injections failed to produce neurological changes can be looked upon as additional controls of the operative procedure.

New method of injecting toxin into an anterior horn: In order to be more accurate in the matter of introducing the toxin into the region of the anterior horns, certain modifications were made in the technique as practiced by us in 1935. The method in use at the present time is as follows: The dura is opened in the midline and one denticulate ligament is cut. Gentle upward traction on the dural flap opposite to the operator rotates the cord, so that one anterolateral quadrant is accessible to him. A tuberculin syringe is substituted for the long capillary pipette with its attached tubing and clamp. The exact amount of toxin to be injected is expressed from a capillary pipette into a watch glass. The droplet of toxin in the watch glass is then very carefully drawn up into a fine needle which is firmly fixed to a tuberculin syringe. Great caution is exercised so as not to aspirate the fluid further than the barrel of the needle, for when this happens the fluid spreads out in the space between the tip of the tuberculin

syringe and the hub of the needle, from which place it cannot be forced back through the needle. The needle measures 1½ inches in length and is no. 25 gauge. The barrel of such a needle holds about 0.003 cc. If one uses more than this amount the fluid reaches the upper end of the needle where it spreads out so that some of it becomes unrecoverable. By having a tightly fitting metal clasp around the plunger of the tuberculin syringe and by carefully rotating the plunger one can accurately fill the barrel of the needle. Indeed with practice it is possible to draw up and to express as little as 0.001 of a cc. two or three times before this amount vanishes. When the needle has been properly filled, the spinal cord is rotated toward the operator and the point of the needle is inserted diagonally into the exposed lateral aspect of the cord until the tip of the needle is in the region of an anterior horn. Then with the utmost gentleness the plunger of the syringe is slowly pressed in. The needle is held firmly in place a full minute and is withdrawn just as carefully as it was inserted. Every possible precaution is taken to avoid trauma to the cord; the point of the needle must be sharp and smooth and must not be moved about; this latter condition is facilitated by the operator resting his hand on the animal during the injection. A typical protocol of an experiment in which the improved technique was used is the following:

July 13, 1937, 2 P.M. Dog 24, fox terrier, female, weighing 6.5 kgm. The skin was shaved and prepared with iodine and alcohol. Under ether anesthesia the dorsal spine and laminae of the third lumbar vertebra were removed and also a small portion of the laminae of the second and fourth lumbar vertebrae. The dura was opened and a denticulate ligament cut. The cord was rotated. There was very little bleeding. We injected 2/1000 of a cc. of tetanus toxin, LYO—2,[1] with a 26-gauge needle. Care was taken not to insert the needle for more than 7 mm., which would almost certainly insure depositing the material

[1] The lyophilized toxin used was prepared by our former associate, Dr. B. Hampil, at the Mulford laboratories (Sharp & Dohme) by dehydrating *in vacuo* a solution of tetanus toxin at −70°F. The residuum was sealed in vacuum tubes and kept at 2°C. until used. Each tube contained 0.2 cc. of toxic veal infusion broth, which was sufficient to kill a dog weighing 20 kilos. It is possible to get the dry residuum in each tube to dissolve in 0.02 cc. of sterile distilled water; thus the M.L.D. for a dog weighing 20 kilos is 0.02 cc. and for a dog weighing 6.5 kilos the M.L.D. is 0.0065 cc.

in the region of the anterior horn. The injection was made from the right side of the cord.

It is very probable that we got scarcely more than 1/1000 of a cc. into the cord because a few minutes elapsed after measuring out the 2/1000 into the watch glass and a little evaporation probably took place before it was aspirated into the needle. Furthermore, some of the material stuck to the needle. It was calculated that 0.0016 cc. of this toxin constituted a quarter of a lethal dose for this animal. No leakage or bleeding was observed when the needle was withdrawn. The wound was closed with silk throughout.

July 14, 6 P.M. The forelegs of this dog seem a little weak. The movements of the head and neck are normal. The hind limbs do not show voluntary movement. They lie in an extended position, but can be very easily flexed at all joints. From time to time the right hind limb goes spontaneously into a series of clonic extensory spasms of mild degree. Tendon and cutaneous reflex irritability is definitely increased in both hind limbs. There is no evidence of head tetanus or of tetanus dolorosus.

July 15, 10.30 A.M. The dog appears very sick, it is lying down, but moves its head normally and can open its mouth freely. The forelegs are used voluntarily and have normal tone. The very slightest sensory stimulus, such as touching a hair on the dog's tail, provokes a series of extensor spasms of both hind limbs. There are 8 or 10 of these clonic jerks at a time. Between spasms the hind limbs lie in a normal position with some flexion at all joints. It is perfectly easy to flex completely both hind limbs at all three joints. A minute ago a house fly lighted on the right thigh, and immediately a series of violent clonic spasms commenced. These spasms involve only the caudal portion of the trunk and both hind limbs. When perfectly quiet, sudden auditory stimuli provoke similar clonic spasms. Tapping the patellar or the Achilles tendon of either hind limb is adequate to produce the same phenomenon. No tetanus dolorosus is observed nor is there the slightest trace of muscular rigidity (Starre) in either hind limb. The temperature per rectum is 99°.

At 3.30 P.M. the dog seemed exceedingly weak, and was carried to an adjoining building for Dr. Abel to examine. Placed on the floor the dog was found to be incontinent and gasping. At this time the hind limbs were completely flaccid and easily flexed. The dog died a minute or two later.

Autopsy was negative except that the site of injection in the cord seemed a trifle swollen and oedematous. The cord was taken out for study and put immediately into 10 per cent formalin solution.

From the foregoing experiments it seems inescapable that one can produce the tactile reflex motor symptoms of tetanus without the slightest evidence of muscular rigidity or tetanus dolorosus. It is interesting to observe that Meyer and Ransom (3) record a spinal cord experiment in which both tetanus dolorosus and reflex motor tetanus occurred without any signs of muscular rigidity.

In this experiment Meyer performed a laminectomy of the first and second lumbar vertebrae in a cat. A drawn out, smoothly rounded, end of a glass tube was pushed through the exposed dura. After some spinal fluid had welled

TABLE I

Control animals with spinal cord injections

DOG	WEIGHT	INJECTIONS			TECHNIQUE	RESULTS
		Material	Volume	Site		
	kilos		*cc.*			
A 2	11.0	Beef extract broth	0.01	Lumbar	Dura opened; syringe method	Animal remained entirely normal
A 7	9.3	Boiled tetanus toxin	0.01	Lumbar	Dura opened; pipette method	Animal remained entirely normal
A 10	13.0	Boiled tetanus toxin	0.01	Lumbar	Dura opened; pipette method	Animal remained entirely normal
35	5.0	Dog serum	0.02	Lumbar	Dura opened; syringe method	Slight weakness right hind limb for one week, thereafter entirely normal
B 9	11.5	Veal infusion broth uninoculated with tetanus toxin	0.01	Cervical	Dura opened; pipette method	Animal remained entirely normal
B 11	13.9	Veal infusion broth uninoculated with tetanus toxin	0.01	Cervical	Dura opened; pipette method	Animal remained entirely normal
B 6	12.0	Boiled tetanus toxin	0.01	Cervical	Dura opened; pipette method	Animal remained entirely normal
B 5	7.0	Veal infusion broth uninoculated with tetanus toxin	0.003	Lumbar	Dura opened; syringe method	Animal remained entirely normal

The results recorded in the above table show that no neurological symptoms occur when innocuous material is injected into the substance of the spinal cord by the technique described in the text.

forth from the tube he injected 0.8 cc. of a solution of tetanus toxin through the tube, which was kept in position thirty minutes to prevent leakage. Thereupon 1 cc. of sodium chloride solution was injected. The subdural injections of these

TABLE II

*Summary of injections of tetanus toxin into the nontransected spinal cord**

DATA	DOG	WEIGHT	DOSE	MATERIAL	VOLUME	SITE	TECHNIQUE	REMARKS ALL OPERATIONS WERE DONE UNDER ETHER ANESTHESIA
		kilos	*m.l.d.*		*cc.*			
3/26/35	B-7	10.0	1/50	Two parts of toxin 22A to one part of broth	0.01	Sixth cervical	Pipette method. Dura opened	Mar. 27: Normal. Mar. 28: Occasional spasms of body and limbs; tactile reflexes of right foreleg are hyperactive. No trismus, no tetanus dolorosus; all joints can be completely flexed. Mar. 29: Almost constant clonic spasms of shoulders and all limbs. Touching right foreleg causes violent spasms; this is not found on touching any other part of the body. No rigidity. Under ether all spasms stop. Autopsy, Apr. 2, negative
10/28/35	A-15	8.2	1/30	0.75 cc. toxin24A to 1.5 cc. dog serum	0.015	Fourth lumbar	Pipette method. Dura not opened	Oct. 29: Normal. Oct. 30: Normal. Oct. 31: Walks with hind limbs spread. Nov. 1: No tetanus dolorosus; both hind limbs go into clonic spasms on slightest cutaneous stimulation; complete relaxation between spasms. Nov. 2: Weaker but clonic jerks of hind limbs continue; no muscular rigidity. Nov. 3: Dead
11/25/35	A-20	9.5	1/162	0.5 cc. toxin 24A to 2.0 cc. dog serum	0.005	Thirdl umbar	Pipette method. Dura not opened	Nov. 26: Normal. Nov. 27: Spares right hind limb; tendon reflexes normal. Nov. 28: Gentle touch of skin or foot of right hind limb causes series of clonic jerks; no rigidity. Nov. 29: Touching right hind limb causes extensor thrusts; frequent clonic spasms of both hind limbs lasting for 2 seconds. Tapping patellar tendon institutes spasms of hind limbs; no tetanus dolorosus, no rigidity; fore limbs normal. Nov.30: Dead
12/ 2/35	A-21	7.3	1/88	0.5 cc. toxin 24A to 2.0 cc. dog serum	0.007	Third lumbar	Pipette method. Dura not opened	Dec. 3: Normal. 4 p.m. Slight muscular tremors. Dec. 4: Clonic jerks. See detailed protocol of text. Dec. 5: Clonic spasms; no rigidity. Dec. 6: Clonic spasms. Dec. 7: Spasms, but no rigidity. Dec. 8: "In extremis." Dec. 9: Dead. Autopsy: Dislocated vertebra and pneumonia

4/30/37	4	7.0	1/35	Veal infusion broth and tetanus toxin 30A in equal parts	0.005	Third and fourth lumbar	Syringe method. Dura opened	May 1: Flaccid paralysis right hind limb; no stiffness. May 2: Tendon reflexes hyperactive; passive flexion of hind limbs at all joints possible. May 3: Clonic spasms following cutaneous stimulation; resistance to passive flexion quickly yields to gentle pressure. May 4: Same. May 5: Much weaker. May 6: Dead. Autopsy: Negative; cord appeared normal
5/11/37	7	5.3	1/50	Two parts of veal infusion broth to one of tetanus toxin 30A	0.004	Third and fourth lumbar	Syringe method. Dura opened	May 12: Right hind limb slightly stiff but can be flexed at all joints; otherwise animal is normal. May 13: Less stiffness; cutaneous stimulaton on left hind limb causes acute flexion. May 14: Constant clonic spasms of both hind limbs; no rigidity. May 15: Dead. Autopsy: Grossly negative; cord normal in appearance, saved for microscopic study
5/12/37	9	4.5	1/50	Two parts of veal infusion broth to one of tetanus toxin 30A	0.003	Third and fourth lumbar	Syringe method. Dura opened	May 13: Normal. May 14: Marked clonic spasms of both hind limbs, these are increased by walking; no rigidity. The sciatic, femoral and obturator nerves were cut on both sides. The clonic jerks of lumbar muscles persisted; forelimbs, neck, and head normal. May 16: Dead. Autopsy: Entirely negative. Cord saved
7/13/37	24	6.5	1/4	Lyophilized toxin 2 used. See Protocol for details	0.002	Third lumbar	Syringe method. Dura opened	July 14: Does not move hind limbs voluntarily but both can be passively flexed. Mild clonic spasms right hind limb. July 15: Looks sick. No head tetanus; fore limbs are flaccid; slight cutaneous stimulus produces clonic spasms of both hind limbs; no rigidity. Dead. Autopsy: Negative except that the cord is oedematous at site of injection. See detailed protocol of text

* The histological changes in the injected spinal cord and a study of the cause of death in all experimental animals will be reported in a forthcoming paper by Dr. W. M. Firor and Dr. A. Lamont.

TABLE II—*Concluded*

DATA	DOG	WEIGHT	DOSE	MATERIAL	VOLUME	SITE	TECHNIQUE	REMARKS ALL OPERATIONS WERE DONE UNDER ETHER ANESTHESIA
		kilos	*m.l.d.*		*cc.*			
7/22/37	27	10.5	1/10	Lyophilized toxin 2	0.004	Third and fourth lumbar	Syringe method. Dura opened	July 23: Seems well except for priapism; tendon reflexes normally active. July 24: Head, neck, and forelimbs normal, hind limbs have fine tremors; attempts at voluntary movements cause violent extensor jerks of hind limbs; no stiffness of any limb; when quiet dog flexes both hind limbs. 4.30 p.m. Extensor spasms of hind limbs increased in severity and last for 2–3 seconds. 9.15 p.m. Dead. Autopsy: Superficial wound infection; muscles and cord not infected; cord a trifle soft at site of injection; viscera normal
7/22/37	28	9.3	1/10	Lyophilized toxin 2	0.003	Third and fourth lumbar	Syringe method. Dura opened	July 23, 9 a.m.: Clonus of hind limbs brought on by slight cutaneous stimulation. 3 p.m. Almost constant severe clonic spasms of hind limbs; extensor thrust when one attempts to flex limbs; this can be overcome and both hind limbs completely flexed, forelimbs entirely normal. 4 p.m. Dead. Autopsy: Entirely negative
9/10/37	41	7.3	1/10	Lyophilized toxin 2	0.004	Sixth and seventh cervical	Syringe method. Dura opened. Cord transected at L3	Sept. 11: Eats well, flaccid paralysis of hind limbs. Sept. 12, 9 a.m. Beginning tactile reflex tetanus, no rigidity; Sept. 13th, 10 a.m. Marked tactile reflex tetanus in both forelimbs, but no stiffness. 2.30 p.m. Unconscious, limbs jerk with each inspiration. 2.45 p.m. Dead; autopsy negative

large amounts of fluid caused the cord to be pressed against the opening of the tube and to act as a stopper for it. After waiting another twenty minutes the glass tube was withdrawn, but practically none of the injected solution escaped from the hole in the dura. The surfaces of the muscles exposed in the operation were touched with anti-toxic solution. Iodoform was applied and the wound sutured. The 0.8 cc. of toxin contained 400,000 + Behring mouse units or six lethal doses.

The operation was done at 12.10 P.M. At midnight no symptoms were yet in evidence. The next morning reflex tetanus was present in both hind legs and in the tail to the extent that these parts responded, by movement, to the slightest touch of a hair. These reflex movements were confined to the parts named. General reflexes were not increased. Tetanus dolorosus appeared in an exaggerated form. The animal cried out at the lightest touch of the hind limbs and had sudden attacks of pain during which it threw itself about and screamed. Such an attack terminated in an extension of the hind legs and of the tail. Between these attacks the muscles were flaccid. Evidence of "Starre" was not obtainable. At 4.30 P.M. the animal was able to move its hind legs and tail voluntarily. The limbs were relaxed except when stimuli of external or internal origin induced convulsive jerks. This reflex excitability was confined to the tail and the hind legs and could be called forth only by touching these parts. The attacks of pain became more violent. 10 P.M. The animal had become weaker and could hardly move about. Respirations were very rapid. Both front and hind limbs were voluntarily movable. There was no "Starre."

The animal was found dead the next morning.

In explanation of this experiment Meyer and Ransom very properly concluded that the protecting pial membrane was injured sufficiently to permit the entrance of some of the 6 lethal doses of toxin into the cord. The authors then go on to refer briefly to their theory that reflex tetanus and the rigidity that were observed in their other experiments, the latter of which was absent here, owe their origin to the action of the toxin on entirely separate centers of the motor apparatus of the cord. It is assumed that in the experiment under consideration the centers responsible for "Starre" had received none of the poison.

It seems certain that the experiments reported in Table II make this explanation by Meyer and Ransom untenable. How would it be possible to place directly in an anterior horn of a lumbar cord $\frac{1}{4}$ of a lethal dose of tetanus toxin and have this toxin reach only the cells responsible for clonic spasms? Surely, if there were separate centers for rigidity (Starre) and clonic spasms in a single horn the

hypodermic needle could not regularly have avoided the one and hit the other.

Experiments resulting in both reflex motor tetanus and muscular rigidity: In the course of our experiments we encountered five animals that showed unmistakable muscular stiffness in addition to the signs of reflex motor tetanus. The protocol of one of these five animals and abstracts of the protocols of the other four follow:

Black puppy, about 5 months old. Weight 5.4 kilos.

December 7, 1936. Under ether anesthesia the spinal cord was transected at the level of the ninth thoracic vertebra.

December 9. A laminectomy was performed at the level of the third lumbar vertebra; the dura was opened, and 1/1000 of a cc. of tetanus toxin, batch 30A, was injected into the right anterior horn by the pipette technique. This was equivalent to 1/60 of a minimal lethal dose.

December 10. The animal is normal except for flaccid paralysis below the level of the transected cord.

December 11. The dog is in good spirits. The bladder and bowels have been emptied. When the dog is held up the right leg and thigh jerk slightly. The lightest tap on the patellar tendon causes extreme contractions of the whole limb followed by a series of clonic jerks. The left hind limb gives a similar but less active reponse. After a period of rest, tapping the left patellar tendon causes a series of clonic spasms of the right limb. While the dog is lying quietly the foot pad of each hind limb is given the lightest possible touch, and this causes extreme flexion of the right hind limb followed by clonic jerks.

December 12. Both hind limbs are stiff; they can be forcibly flexed but this attempt causes clonic jerks.

December 13. The animal's condition is the same as yesterday. Both hind limbs are held stiffly in extension. The dog died during the night. The autopsy was negative.

The *second dog* also had a transection of the dorsal cord, and three months later 3/1000 of a cc. of tetanus toxin, batch 30 A, was injected into the lumbar cord. Two days later there was beginning stiffness of the right hind limb and hyperactive reflexes of the left. The next day both limbs were stiff and showed unmistakable reflex motor tetanus. The following day this note was made: "The hind limbs are kept in extension and are really stiff but both can be forcibly flexed at all joints. There are constant tremors and frequent clonic spasms of both hind limbs. The slightest tactile stimulation serves to elicit a series of clonic jerks." The dog continued in this state until it died a week later. The autopsy was negative except for extensive cystitis.

The *third dog* was one in which an unsuccessful attempt to introduce tetanus toxin into an anterior horn of the lumbar cord had been made six weeks previous to the successful injection. The latter amounted to 1/200 of a minimal lethal

dose and was made caudad to the former operation. Three days later frequent spontaneous clonic jerks of the posterior half of the body appeared. Similar clonic spasms were produced by the slightest possible cutaneous stimulation of the left hind footpad. This tactile reflex tetanus continued until the dog was killed a week later but the intensity of the spasms diminished during the last two days of life. Stiffness of the hind limbs was first noted on the fifth postoperative day. This symptom increased in severity so that finally it was impossible to flex any joints of the hind limbs. There was no sign of tetanus dolorosus at any time. The autopsy showed a very dense scar at the site of the old operation. The muscles were firmly adherent to the spinal cord, which was dilated below the point of constriction.

The *fourth dog* was one that received a trifle less than 1/1000 of a cc. of toxin in the lumbar cord. This was equivalent to about 1/100 of a M.L.D. Two days later there was beginning stiffness in the left hind limb and reflex motor tetanus in the right one. The next day these symptoms persisted and the wound appeared to be infected. Thereafter the stiffness of both hind limbs increased, going on to unyielding rigidity. Coincidently the wound infection became progressively worse. The animal was killed a week after the operation. There had been no signs of tetanus dolorosus. The autopsy showed an abscess extending well down into the muscles, making them adherent to the cord. The adhesions were so dense that the cord was constricted. There was a pocket of yellow watery fluid about the distal part of the cord. The leptomeninges did not appear to be acutely inflamed.

The experiment carried out on the *fifth dog* differed *in several ways* from all the others we have done. The laminectomy extended from the second lumbar vertebra to the sacrum. Four injections were made at separate points into the gray matter instead of a single one. These injections were made with the fine tip of a glass pipette. A total of 7/1000 of a cc. of toxin (1/50 of a M.L.D.) was injected. To this toxin a small amount of carefully washed sterile charcoal had been added. The right hind limb had a flaccid paralysis immediately after the operation. Three days later this limb began to be stiff, and the stiffness increased steadily. It was noted on the fifth postoperative day that the thigh muscles were constantly twitching. The left limb, however, had hyperactive reflexes the day after operation; these persisted and increased, giving signs of a moderately severe reflex motor tetanus. Stiffness began in this limb on the fourth postoperative day but the limb never became rigid like the right hind limb. The animal died nine days after operation. The autopsy showed cystic degeneration in the gray matter at the site of the injections. Otherwise the necropsy was negative.

Comment: It will be observed from the abstracts of the five preceding experiments that there was evidence of severe trauma to the spinal cord of each dog. In two instances the injury was in the form of transection, in two others there was a constricting band of scar

tissue densely adherent to the cord. In the fifth dog there was an area of cystic degeneration in the gray matter resulting from the multiple punctures with a glass pipette. These observations confirm our belief that the rigidity, noticed by previous investigators after injections of tetanus toxin into the spinal cords of rabbits and cats, was due to a preceding or concomitant injury of the cord. As a matter of fact in all spinal cord experiments reported by Meyer and his associates, except the five quoted in the first part of this paper, the investigators transected the cord cephalad to the site of injection before introducing the toxin. No one can claim that the physiological response of the distal segment of a transected cord is the same as the response of an intact cord. We feel, therefore, that the rigidity occurring after the introduction of tetanus toxin into an anterior horn of a transected cord is an unnatural form of tetanus due to the transection. When, however, the level of the transection is caudad to the point of injection, the reaction to the toxin is the same as that of an intact cord. This is illustrated in the last experiment recorded in Table II, for in this experiment the toxin was placed in the cervical cord and the transection was made at the level of the third lumbar segment. The animal subsequently showed well developed tactile reflex tetanus without the slightest evidence of muscular rigidity.

DISCUSSION

In reviewing the literature, it is surprising to find how seldom injections of tetanus toxin have been made into the intact cord. One wonders why Meyer or some of his associates did not repeat the experiment, abstracted above, in which he obtained reflex motor tetanus and tetanus dolorosus without muscular rigidity. In the five experiments on the intact cord reported by Meyer and Ransom, the occurrence of that grave complication, tetanus dolorosus, detracts from their value. Furthermore, the brief incubation period, 2 hours, and the early death of the animals, 10–20 hours, and the fact that the reflex motor symptoms were but little in evidence indicate that these experiments do not merit the significance that has been accorded them.

From the eleven experiments reported in Table II, it seems ines-

capable that one can produce the tactile reflex motor symptoms of tetanus without the slightest evidence of muscular rigidity or tetanus dolorosus. To accomplish this, one has only to deposit in an anterior horn of the spinal cord a suitable quantity of tetanus toxin. In our experiments this quantity varied from $\frac{1}{4}$ to 1/162 of the calculated minimal lethal dose and the volume of the injected material varied from as little as 2/1000 to 15/1000 of a cc. Anyone who has ever seen the real muscular rigidity of local or generalized tetanus has observed a hard, unyielding spasticity that could not be confused, by any stretch of the imagination, with the spontaneously occurring movements or the induced reflex extensor thrusts that were seen in our experimental animals. These reflex spasms were easily overcome by gentle, steady pressure and in every animal it was always possible to flex completely all three joints of the affected limbs. Furthermore, these reflex thrusts were abolished in the first stage of ether anesthesia, whereas this is not true of the rigidity of descending and local tetanus.

The fact that we could not produce muscular rigidity on injecting the toxin into an anterior horn of the non-transected cervical and lumbar cord, may be adduced as evidence in the support of our belief (4) that the cause of muscular rigidity, as seen in both local and general tetanus, is not to be sought in an action of the toxin on special centers, but in its action on the voluntary muscles.

Another observation made upon our experimental animals is the presence of a distinct latent period between the deposition of the toxin in the anterior horns and the development of reflex motor tetanus. This period was from 28 hours in the dog receiving $\frac{1}{4}$ of a M.L.D. to 72 hours in the animal receiving 1/162 of a M.L.D. Our experiments show that even after injecting an anterior horn with such relatively enormous[2] doses as $\frac{1}{4}$ of a minimal lethal dose there is always an incubation period of many hours. It is well known that the appearance of local tetanus following the intramuscular injection

[2] This dosage is indeed enormous when it is considered how sensitive central neurons are to tetanus toxin. In our earlier unpublished studies of 1935 we frequently observed most extreme forms of tactile reflex hyperexcitability conjoined with tetanus dolorosus following the injection of 1/300 of a lethal dose into the dorsal part of the cord.

of toxin is likewise preceded by a period of incubation and that this period is shortened as the locally employed dose is increased. The toxin therefore requires time for the development of its action, irrespective of whether its point of attack is the central neurons or their terminals. There is every reason to believe that in natural tetanus the central incubation time is fully as long as the peripheral one; the evidence, indeed, points to the conclusion that in all but the most rapidly fatal cases of descending tetanus, it lags behind the latter in the first few days of the disease.

Another observation made in this series of spinal cord injections is the complete absence of evidence that tetanus toxin passes upward in the spinal cord. Even when $\frac{1}{4}$ of a minimal lethal dose was placed directly in the gray matter of the lumbar cord, the signs of tetanus were limited strictly to the site of injection. Surely, if tetanus toxin normally passes up the cord one would expect it to do so in at least one of the eleven dogs described in Table II. The objection that experimental introduction of toxin into the cord is a wide departure from its deposition there by the blood stream in naturally occurring tetanus does not withstand close scrutiny. For it is abundantly clear from our experiments that the toxin when artificially deposited in the anterior horns of the cord causes exactly the same central nervous system manifestations that are seen in clinical tetanus, and no others. Furthermore, four of our dogs lived from five to seven days, thus allowing ample time for the upward passage of toxin, but in not a single instance was there any evidence of this phenomenon. Upon reflection one realizes that the gray matter of the spinal cord has a rich network of capillaries. It seems inconceivable that the molecules of tetanus toxin could work their way through this maze of blood vessels, escape absorption, and travel 20 or 30 centimeters to the medulla, in the absence of any mechanism like the intercommunicating lymphatic networks present in other parts of the body.

SUMMARY

The spinal cord experiments of Meyer and his associates are critically reviewed. A technique for injecting an anterior horn of the spinal cord with tetanus toxin is described. By this method it is possible to produce pure tactile reflex motor tetanus without accom-

panying tetanus dolorosus or muscular rigidity. Abstracts are given of experiments on eleven dogs in which this was accomplished and also the abstracts of eight control experiments in which innocuous substances were similarly injected. Abstracts of five additional experiments are offered to show that the development of rigidity, following the injection of tetanus toxin into the spinal cord, occurs when the cord is transected or subjected to other forms of trauma. It is pointed out that in all of our spinal cord injections there was an incubation period varying from 26 to 72 hours. Finally, reasons are given to show the extreme improbability of an upward passage or diffusion of tetanus toxin within the cord.

The authors take this opportunity to acknowledge their indebtedness to Dr. John J. Abel. It is a pleasure to thank him for his constant assistance and friendly council.

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ON THE OUTPUT OF SUGAR AND POTASSIUM BY THE TURTLE'S SURVIVING LIVER

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Of the many factors known to be concerned in the peripheral nervous control of glandular activity very little is known as to the order in which they come into play or their relative importance one to another. Our attempts to gain new knowledge in this field are beset with the well nigh insuperable difficulty of establishing adequate controls. For in the intact body where the organ is supplied with the blood uncontrolled, "spontaneous" changes may occur from moment to moment in the concentrations of hormones, enzymes, and the various metabolites, to say nothing of chance nervous discharges into the organ. On the other hand, if the organ is isolated, it survives on a solution which cannot fully take the place of properly aerated blood supplied under proper physical conditions. Nevertheless, in the latter case one does have complete control of the conditions external to the organ; what then still remains uncontrolled are the intrinsic factors of the organ itself, which as the sequel will show may cause many a doubt in the interpretation of results.

The present paper reports the results of an experimental study on the isolated turtle's liver surviving on a modified Tyrode's solution perfused through the portal vein at a steady head of pressure. The object was to determine the changes in the output of sugar and potassium from the liver under various conditions.

Eiger (1916) observed that the isolated perfused liver of the turtle upon stimulation of the vagi took on an increased amount of glycogen compared to the unstimulated organ. Snyder, Wells and Culley (1923) demonstrated on a similar preparation a decrease in the amount of sugar in the outflow per minute-volume, upon stimulation of the vagi and upon the addition of pilocarpin to the perfusate. The

action of pilocarpin was antagonized by atropin. Snyder (1937) could show that a vagus stuff comes off from the liver following vagal nerve stimulation. From such results as these there can be no doubt that the vagi influence the carbohydrate-storing properties of the liver. But the mechanisms involved may be influenced variously, as are those concerned with the glycolytic function of the sympathetic nerves.

Following the demonstration by Snyder and Martin (1922) that the character of the response of the turtle's hepatic vasculature to epinephrine depended upon the pH of the perfusing fluid, it was shown by Snyder, Martin and Levin (1922) and again by Snyder, Wells and Culley (1923) that changes in the pH of the perfusing fluid not only modify markedly the sugar output of the liver, per minute-volume outflow, but that they also modify the apparent glycolytic power of minimal doses of epinephrine added to the perfusate. Between limits it was found that the output of sugar increased with the increase in H-ion concentration. The amount of increase, with pH at 7.4 and less, is further increased by adding minimal doses of epinephrine to the perfusate; the decrease in sugar output at the higher pH levels is further decreased likewise upon adding minimal doses of epinephrine to the perfusate. This is not only a clear case of epinephrine reversal, but also gives a clue as to the probable cause in general of this phenomenon. The action of acetyl β-methyl choline upon the rate of outflow from the liver likewise was shown to depend upon the pH of the perfusing fluid, increasing it when the pH was set at 7.0, decreasing it when the pH was at 7.5 (Snyder, 1937).

From the foregoing it is obvious that in analyzing the changes suffered by any constituent of the blood or blood-substitute passing through an organ, mere determination of the concentration of that substance before and after is not enough. The minute-volume of outflow also should be recorded. Gain or loss in the substance analyzed then may be put on a minute-volume-output basis. To complete the data necessary for a final statement as to the fate of the substance, analyses should finally be made of the organ itself as to its content in water as well as the particular substance under investigation.

The idea that at least some nerves may produce their effects not

directly but by intermediate chemical transmission was clearly stated by W. H. Howell as early as 1906, when in a discussion of the nature of the accelerator and inhibitory nerve of the heart he wrote: "The author was led to suggest some years ago that the inhibitory influence of the sympathetic upon the heart may be exerted through some *intermediate* effect upon the potassium and calcium compounds on the heart tissues" (p. 281, 1906), and "It seems justifiable to assume as a provisional hypothesis that the vagus inhibition takes place because the nerve impulses effect a sudden increase in the amount of diffusible potassium compounds in the heart tissue. . . . It is quite conceivable therefore that *the action of the vagus impulses consists primarily in augmenting such dissociation and thus setting free an inhibitory substance* . . ." (p. 291, 1906; italics, present authors').

Of late a number of workers have been studying the potassium of the blood as it is affected by various treatments. The remarks in the above paragraph are applicable to all phases of such studies. Howell and Duke showed in 1908 an increase in the K content of a perfusing solution circulated through the mammalian heart during faradic tetanization of the vagi; they then looked for a possible increase in Ca-ions in a similar heart preparation, accelerated by sympathetic nerve stimulation, but failed to find it. Lánczos (1935), however, by means of a well-planned series of experiments on frog hearts, was able to demonstrate that Ca-ions are liberated during stimulation of the attached sympathetic nerves, thus confirming the suggestion of Howell and Duke. On the other hand the finding of the latter named authors of increased potassium, upon vagal stimulation, was confirmed by Lehnartz (1936), although he does not consider K as being the true transmitter of vagal impulses. Fischer (1936) interprets his own observations on eel- and turtle-hearts as indicating that upon stimulation each kind of vagotropic nerve gives rise to a specific kind of chemical substance to act as transmitter, K being one of them.

Adrenalin, when injected into the perfused liver of the cat, was found by D'Silva (1936) to cause a momentary increase in the K content of the outflow; continued perfusion with blood liberated K for longer periods (*ca.* 6 minutes) and subsequent injection of adrenalin at 5- to 10-minute intervals liberated comparatively large amounts

of K. After ergotoxine no increase in K was observed following adrenalin infusion. By means of a number of control experiment the author finds that the increase in the K content of blood serum from intact animals, as observed by himself (1933–34) and by Schwartz (1935) upon injection of adrenalin, is due entirely to the K liberated by the liver. Camp and Higgins (1936) observed that all the reactions obtained by the injection of physiological doses of epinephrine into dogs could be evoked by the injection of small doses of KCl (0.2 ml. of a 5 per cent solution per kilogram body weight); they concluded that one of the chief functions of the adrenal glands is to maintain a constant distribution of K in the body.

The importance of the question, as to what extent apparent changes in the concentration of substances in the blood depend upon the absorption or release of water by the tissues, is exemplified by the experiments of Geiger (1931), who in a study of the mechanism of glycogen mobilization in the frog's liver observed that the liver loses water under the influence of adrenalin and histamine but could not find that this loss of water was due to vasoconstriction or to increase of intravenous or intracapillary osmosis. Geiger adopts the view of Lesser that the loss of water is due to a specific action of adrenalin on the colloids of the liver cells, causing them to give up water or to inhibit their absorption of water, and finally concludes that the dehydration is not a merely concomitant process, but rather the cause of glycogenolysis.

This brief review of the literature is sufficient to indicate that the final elucidation of the problem of peripheral control will only be found after much systematic work has been done. It was decided therefore to begin with a series of experiments in which the conditions are simplified to the utmost. Knowing the exact composition of the inflowing fluid, any changes it suffers in passing through an organ can be determined by appropriate analyses of the outflow. The differences in composition between inflow and outflow fluids during control periods, during the periods when additions or subtractions are made to the inflowing fluid, and during electrical stimulation of attached nerves may then be determined *ad libitum*.

The present paper deals with the results of a series of such experiments in which the sugar and potassium content of the outflow was

compared with that of the inflowing perfusate sent through the organ by way of the portal vein, (1) under constant conditions, (2) following peripheral nerve stimulation, (3) following addition of autonomomimetic drugs added to the inflowing fluids.

The usual procedure, after pithing brain and cervical medulla and exposing viscera on the ventral aspect was as follows: (1) perfusion from a reservoir of Tyrode's solution is established through a bifurcated pathway into the liver by way of portal vein and into the heart by way of the ascending vena cava. (2) The two organs are then isolated from the body and from one another by (*a*) ligating the descending venae cavae, securing an outflow cannula either in the median aorta and tying off the other aortic outlets, or in the atrioventricular foramen, and (*b*) ligating the left lobe of the liver across the isthmus and then (if by this time the outflow from the right lobe is quite free from blood) by securing an outflow cannula in the hepatic vein and tying off the latter at its junction with the sinus venosus. (3) The heart is included in the experiment on the liver in order to have an immediate external index of the efficiency of solutions, drugs and electrical stimulation of nerves. Therefore, either ventricle or auricle or both are finally attached to levers for graphic recording, the end of the outflow tube from the heart being set a little above the heart level of the stump of the hepatic vein. (4) The head of inflow pressure and rate of volume flow are adjusted now so that the irrigation is optimal for the heart action and to maintain, under normal nonstimulating conditions, an outflow from the liver amounting to about 3 ml. per minute. (5) If nerves are to be stimulated they are now finally prepared, electrodes fixed in position and precautions taken against drying and short circuits. (6) The liver outflow is caught in an automatically dumping and recording vessel which is set to collect the outflow usually in 8 ml. volumes. (7) When all the above has been accomplished and the perfusion has been continued at a steady state for a half hour or longer to insure the establishment of an equilibrium between the solutes of the perfusing solution and the tissues of the organs perfused, the collection of the samples (in graduated centrifuge tubes) is begun and continued to the end of the experiment. After their volumes have been recorded the samples are then prepared for analysis.

For the sugar analyses the method of Shaffer, Hartmann and Somogyi was employed. For the potassium determinations we tried at first that of Breh and Gaebler but finding the method of Hubbard (1933, a modification of the Kramer and Tisdall method) much more reliable for our material, we finally adopted that. (For the additional modifications which Hubbard has recommended we are indebted to Dr. Anna Baetjer to whom they were originally sent by personal communication.) Our perfusates contained no protein and by the method we used very little passed out of the liver into our samples. The samples however were immediately centrifuged in order to separate the few blood cells. Portions were then taken separately for the analyses. While we could neglect incineration to get rid of the protein for the potassium analysis, nevertheless, for the sugar determinations the portions were put through the usual Folin-Wu process before going further. Of course both methods were carefully calibrated on solutions of known compositions in order to determine their limits of accuracy, which we found to be close to the limits set by their authors.

Since Tyrode's solution is the more suitable solution for surviving strips of intestine, it probably more nearly approaches the composition of portal blood than does a simple Ringer. It was therefore chosen for the liver perfusion fluid, making it up with 0.63 per cent NaCl and saturation with a $CO_2 + O_2$ gas mixture, CO_2 being added until the desired pH was established. In the later experiments gum acacia was added to 1.4 per cent; with this in the inflow less of the tissue proteins appeared in the outflow from the liver, and the heart survival was excellent.

The aim of the experiments being to observe the changes the sugar and potassium in the perfusion fluid suffered on its passage through the liver, the differences in the substances found by analysis were reduced to denominations of gain or loss by weight per minute-volume outflow. The differences observed in these gains and losses during the progress of an experiment were then finally expressed in terms of percentage change in gain or loss in minute-volume outflow.

RESULTS

In the first place it was found that the changes in percental gain or loss during control experiments, lasting from 20 minutes to an

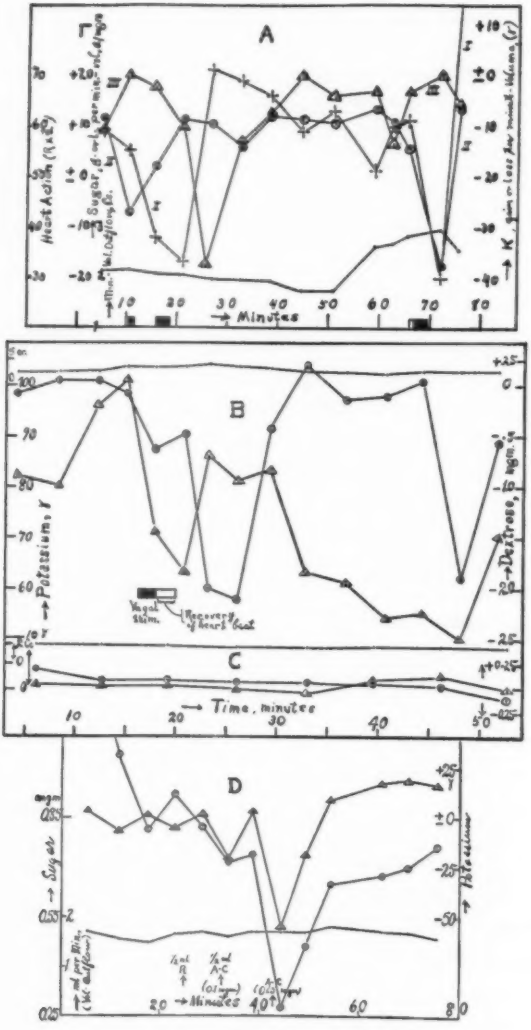
A
B
C
D
Minutes
Time, minutes
Potassium, γ
Distress
Sugar
Vagal stim.
Recovery of heart beat

FIG. 1

hour, sometimes could be very large. This occurred in spite of all our efforts to maintain all the conditions of the perfusion quite constant. The maximum changes occurring during the whole periods of such control experiments will be found in the upper part of Table I. From such results it is quite clear that researches of this kind should include controls covering periods equal to those of the experiments themselves.

The maximum changes following faradic stimulation of the vagi in many cases were well within the magnitudes found in the controls. In two cases however for the sugar and in one case for the potassium they were far greater than in any of the controls. Furthermore, the great majority of them were in a negative direction, indicating that following vagal stimulation the output of sugar and potassium from the liver for a few minutes is reduced. For this evidence see the second part of the table, and the graph-groups A, B and C in figure 1.

Similarly the addition of acetyl β-methyl choline to the perfusing solution reduced the sugar and potassium minute-volume output of the liver although the magnitude of the changes also was usually within the possible changes occurring among the controls. In one case the reduction in the sugar output was more than five times

FIG. 1. ILLUSTRATING THE RESULTS OF FOUR KINDS OF EXPERIMENTS

The upper groups of curves, A and B, plot the detailed data for two experiments in which the vagal nerves had been tetanized with induced current for brief periods. The C group of curves gives the data of a control experiment. The D group of curves gives the data when acetyl β-methyl choline was injected into the portal vein. The symbols used are the same throughout: the plots in circles (⊙, ⊙, ⊙), give the gain or loss in sugar per minute-volume of the hepatic outflow; the plots in triangles (△, △, △) the gain or loss in potassium per minute-volume; the plots in naked dots (•, •, •) give the actual minute-volume hepatic-outflow in ml. for each sample collected; the plots in crosses (X, X, X) in curve I of group A give the mean heart action (rate, times, extent of lever lift) during the time each sample was collected.

The times of vagal stimulation are shown by the black quadrilaterals set in along the time axis; the latent and recovery periods, as indicated by the action of the heart, are shown by the unshaded portions of the quadrilaterals.

The abscissae plot the time duration of the experiments in minutes. The dimensions of other quantities are plotted along lateral ordinates. The sugar of the first sample in the D group amounted to 1.42 mgm.; to save space it is not shown.

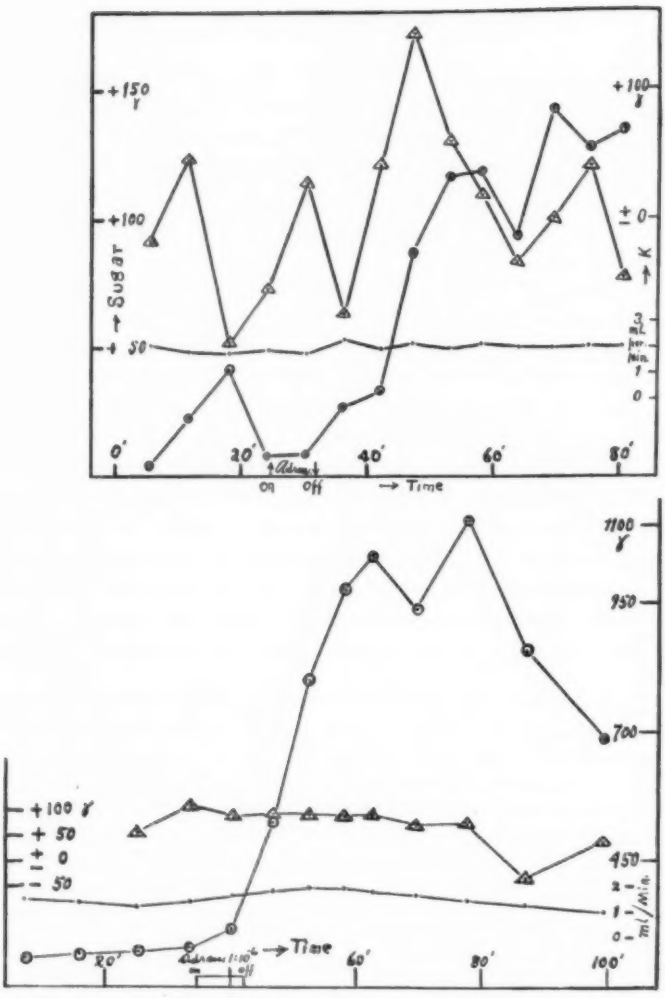

FIG. 2. RESULTS OF TWO EXPERIMENTS IN WHICH ADRENALIN WAS ADDED TO THE PERFUSION SOLUTION FOR A SHORT TIME IN EACH CASE

The symbols used in plotting the data are the same as those used in figure 1. The upper group of curves gives the results of the only case out of five experiments in which a marked increase in K followed perfusion with adrenalin; the interpretation of this rise is made doubtful by the marked oscillation in the K content before the adrenalin was given.

TABLE I

Showing maximum variations in the percental changes in the gain or loss (+ or −) in sugar and potassium in the minute-volume outflow from the turtle's liver under various conditions

KIND OF EXPERIMENT	DATE	SUGAR	K	REMARKS
I. Controls	February 11, 1937	−150	−500	pH 7.45; no acacia
		+518	+178	
	May 1, 1937	−60	−200	pH 7.30; + acacia + atropin
		−146	+417	
	May 6, 1937	−121	−66	pH 7.35; no acacia
		+251	+61	
	Mean	+49	−18	
II. Following vagal n. stim., faradic	November 7, 1936	+76	−73	pH 7.8
	February 13, 1937 (1)	−551	−141	pH 7.45
	(2)	−550	+98	
	February 27, 1937 (1)	−2310	−68	pH 7.35
	(2)	−278	−90	
	March 12, 1937 (1)	−19	−11	pH 7.55; perfusion solution eserinized
	(2)	−69	−1040	
	(3)	+29	−116	
	March 29, 1937	−2258	−38	pH 7.45
	Mean	−659	−164	
III. Following acetyl β-methyl choline injection	December 11, 1937 (1)	−14	−30	pH 7.50; perfusion solution eserinized
	(2)	−41	−11	
	November 21, 1936 (1)		−56	pH 7.40; perfusion solution eserinized
	(2)		−200	
	February 4, 1937	−692	−284	pH 7.5
	June 8, 1937	−69	−125	pH 7.20; + acacia
	June 18, 1937	−28	+100	pH 7.35
	July 13, 1937 (1)	−16	−940	pH 7.40; + acacia
	(2)	−63	−1710	
	Mean	−132	−362	
IV. Following adrenalin injection	May 6, 1937	+294	−139	pH 7.35; + acacia
	May 15, 1937	+405	+301	pH 7.80; + acacia
	June 8, 1937	+850	−20	pH 7.20; + acacia
	June 16, 1937	+103	+63	pH 7.60; + acacia
	June 18, 1937	+553	−435	pH 7.35
	Mean	+441	−46	

greater than in any of the controls; in another case the reduction of the output in K was more than five times greater than in any of the controls. See graph-group D in figure 1 for the detailed analyses of this experiment, and section III of the table for the maximum percental changes in the various experiments.

On the other hand, when adrenalin-HCl (Parke-Davis Company) was added to the perfusate for a short period the sugar showed always a gain sooner or later in the outflow, while the potassium showed sometimes a gain and sometimes a loss, as appears in section IV of the table. The detailed progress of events occurring in two experiments are shown in figure 2.

SUMMARY AND CONCLUSIONS

1. Methods are described for the isolation of the turtle's liver and heart for separate perfusion on an artificial solution to maintain their survival for long periods of time. The heart is kept surviving to serve as a constant external indicator of the efficiency of the solutions used and of any drugs one may wish to add thereto, and also of the efficiency of electrical stimulation applied to the cervical vagal trunks.

2. During a series of such perfusion experiments consecutive samples of the liver outflow were collected, their volumes recorded and the minute-volume outflow determined. The samples were then analyzed for their content of sugar and potassium and compared with the content of those substances in the perfusion fluid. The gain or loss of sugar and potassium in the outflow over that of the inflow thus determined was finally reduced to a minute-volume basis.

3. Among the outstanding results of the investigation are the following:

a. During prolonged control periods marked changes occurred in the gain or loss of both sugar and potassium per minute-volume. It was concluded that these changes were due to intrinsic factors rather than to any uncontrolled external factors.

b. Following vagal stimulation, or the addition to the perfusate of acetyl β-methyl choline, both the sugar and the potassium per minute-volume output tended to be diminished. In a few cases the extent of the reduction was very marked. There was no evidence that the K-output under parasympathetic stimulation is increased.

c. Upon the introduction of adrenalin-HCl into the portal inflow for short periods of time the output of sugar in the outflow was usually increased, sometimes quite markedly. The potassium, however, appeared to be unaffected by the adrenalin, a finding which fails to confirm the results of D'Silva on the cat's liver.

d. The weights of the organs before and after the experiments and specific gravities of the fluids concerned were not taken, and no record was made of the total volumes of fluid entering and leaving the livers. Therefore no exact statement can be made as to what extent water absorption or liberation by the hepatic cells affected the concentrations of substances in the outflow.

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STUDIES ON EXPERIMENTAL HYPOPHYSECTOMY IN DOGS

III. Somatic, Mental and Glandular Effects

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Our first report (1925) (1) on the total removal of the hypophysis in dogs went to show that the hypophysis was not essential to the maintenance of life.[1] This, our third and greatly delayed publication on this subject, is concerned with the somatic, mental and sexual changes induced by the removal of this gland during the various periods of life. Some of these results have been published in Volume I of *Engelbach's Endocrine Medicine* (2) and in Volume II of the *Memoirs of the University of California* (3). When our studies were begun there were two diametrically opposite conclusions concerning the relationship of the hypophysis to the maintenance of life: (1) Aschner (4) had shown that it was not essential, while (2) Paulesco, Cushing and his coworkers had concluded that animals could not survive its total extirpation.

To Cushing (12) the explanation of Aschner's survivals lay in incomplete extirpations. He was further convinced that a definite train of symptoms marked the few remaining hours or days of life in a totally hypophysectomized dog. These—loss of weight, subnormal temperature, increasing lethargy—he chose to group as a syndrome—cachexia hypophyseopriva. Aschner was never able to understand the differences. It was in the hope of finding the cause of these discordant results that our experiments were undertaken.

[1] We wish to take this opportunity of correcting our mistake in stating that Houssay had found the hypophysis to be essential to life. He—using the buccal route—had clearly stated that animals survived its total extirpation.

That the one (Cushing) used an intracranial, the other (Aschner) a transbuccal route did not then appear to be of any particular importance. To one with a surgical bent a clean temporal route was always superior to one through the roof of the mouth teeming with organisms. However, infections could not be said to have caused any of Aschner's results. Nor could it be claimed that total extirpation of the hypophysis should be any more difficult or uncertain by the buccal approach: on the contrary, the reverse was more probably true. It was not long before we found that the animals did survive total removal of the hypophysis by the intracranial route; it was next learned that the number of survivals was in direct proportion to the ease and rapidity with which the hypophysis could be extirpated, *i.e.*, the avoidance of trauma to the brain during retraction of the temporal lobe to expose the hypophysis. To better attain these ends two operative improvements were developed: (1) the brain was shrunken by injecting hypertonic salt intracranially, and (2) by inverting the head so that the brain gravitated from the bone of the skull and required almost no retraction. Only by the use of these expedients was it possible to carry out the operation successfully on young puppies (3 to 4 weeks old). When adept, the operator should be able to complete the operation in twenty minutes or less and to require less than two minutes for actual retraction of the temporal lobe. Our results, therefore, were in absolute accord with those of Aschner. The so-called "cachexia hypophyseopriva" syndrome (in animals) proved to be nothing more than the manifestations of approaching death from intracranial pressure, pneumonia or other causes.

In this report are included the ultimate somatic and sexual changes from a series of dogs subjected to partial and total hypophysectomy by this method. To insure, when desired, the elimination of cellular remnants, the infundibular attachment to the base of the brain was cauterized with the red hot bulbous tip of a metal probe. At least it can be said that the removal of the hypophysis and its cellular prolongations along the stalk have been even more complete than was possible by mechanical means used exclusively up to that time.

After reviewing our results, we find nothing to detract from and little to add to the admirable contributions of Aschner. There are

few experimental studies that can rank higher in accuracy and thoroughness, and few will better stand the test of time. He antedated the excellent experimental results of Smith (1916) (6) and Allen (1916) (13) in tadpoles, and his findings for the higher animals were essentially the same as those attained in the lower forms.[2]

EXPERIMENTS

Most of the puppies were operated upon when three, four, or at the most, six or eight weeks old. The younger the animal the more profound is the disturbance of development. The operative risks are much greater (by the intracranial route) in very young puppies because the brain is much softer and consequently more easily traumatized. Moreover, there is less intracranial room because the amount of cerebrospinal fluid is relatively less and sufficient traction to expose the hypophysis is impossible without great injury to the brain. By a preliminary injection of hypertonic salt solution, intravenously, the volume of the brain is reduced to such a degree that undue trauma to it is avoided. The procedure of rotating the head nearly 180° is also important in that it permits the brain to fall away and thus reduces the retraction to a minimum. When cauterization of the infundibular region is employed to destroy all hypophyseal cells, the operator must be careful not to apply it too severely, for too much heat will quickly cause death from cerebral oedema.

Because of the excessive mortality when dogs are kept in the laboratory the puppies were either returned immediately after the operation to the original litter and put on a roof garden in pleasant weather, or kept by interested friends in more ideal surroundings in the city. Puppies from the same litter were always used as controls and were kept with the hypophysectomized puppies as long as possible. Similar feeding conditions were maintained. Most of the puppies were mongrels, but only litter mates and of approximately the same size were selected as controls. They were photographed, weighed and x-rayed at frequent intervals. When one of the hypophysectomized

[2] For a more complete review of experimental studies of the hypophysis especially in lower animals, the reader is referred to the splendid publications of Evans (3), and of Van Dyke, *The Physiology and Pharmacology of the Pituitary Body*, The University of Chicago Press, 1936.

animals died, a control, when possible, was sacrificed in order that accurate comparative gross and microscopic studies could be made of all the organs. The hypothalamus of the hypophysectomized animals was serially sectioned in order to permit search for hypophyseal cells.

The protocols with charts and illustrations that have been selected for this report concern eight puppies with their controls. Each, except two (H^{32} and H^{33}), on study of serial sections of the base of the brain, was found to have undergone a total hypophysectomy, that is, complete removal of anterior and posterior lobes, but minute bits of pars tuberalis were found attached to the base of the brain in H^{16}, H^{76}, H^{92} and H^{99}. In puppies H^{32} and H^{33} there were small macroscopic remnants of anterior lobe cells, so that these animals will serve as examples of subtotal hypophysectomy.

The rate of growth was determined by roentgenograms at frequent intervals of the skull, and the right hind limb in particular. The tube was uniformly placed six feet from the animal and by measuring on the roentgenogram the distance from inion to nasion, and the length of the femur and the tibia, a fairly accurate index of the skeletal growth was obtained. From the roentgenograms it was also possible to determine the condition of the epiphyseal lines and their time of closure; the x-rays of the skull showed the time of appearance of second dentition. In the tables, growth is recorded by the length of the skull and by the sum of the lengths of the femur and tibia.

Protocols of experiments

I. Total hypophysectomy. H^1 (total hypophysectomy) and H^2 (control). A male, fox terrier puppy (H^1) was totally hypophysectomized June 29, 1924, when exactly three weeks old. The operation was performed under ether anesthesia and before the adoption of intravenous hypertonic salt solution to shrink the brain. The traumatized temporal lobe was partially resected. The puppy's weight at the time of the operation was 1.2 kgs., while the male litter mate control (H^2) weighed 1.0 kg.

First series of x-rays of H^1 was taken August 24, 1924 (eight weeks after the hypophysectomy). Measurements of the control are in parentheses.

	H^ and control 2 mos.	
Humerus	4.5 (8.2)	Small epiphyses at both ends (larger epiphyses in control).
Ulna	4.7 (8.2)	
Radius	4.5 (7.8)	
Femur	4.4 (9.2)	Small epiphyses. (Larger epiphyses in control.)
Tibia		
Fibula	4.8 (8.2)	
Pelvis	4.4 (7)	Ischium still divided on each side (United in control).
Metacarpal	1.7 (3.5)	Tiny epiphyses. (Large in control) no union.
Metatarsal	1.5 (3.8)	Only two bones—astragalus and another (all well developed big bones in control).
Skull (Nasion Inion)	6.6 (9)	
Teeth	Baby teeth in operated puppy (second teeth formed in control, but not erupted).	
Vertebrae	Thin epiphyseal plates (traces of fissure still present in control).	
Tarsals	Only two small tarsal bones in operated puppy (well developed in control).	
Carpals	Five tiny carpals in operated puppy (well developed in control).	
Penis	No bone in penis of operated puppy (but slight calcification in control). No signs of union of any epiphyses in either operated or control puppy.	

Second series of x-rays was taken October 30, 1924 (18 weeks after hypophysectomy). The following measurements were made (control measurements are in parentheses).

Humerus	4.7 + epiphyses = 5.7 (11.00)	
Ulna	5.8 (12.5)	Fairly large epiphyses in hypophysectomized animal, but shapeless. (Much larger and well formed in control.)
Radius	5 (11.0)	
Femur	5.8 (12.4)	
Tibia		
Fibula	5.4 (11.5)	
Pelvis	Still ununited (well united).	
Metacarpal	2.0 (4.4)	
Metatarsal	2.2 (5.3)	
Skull (Nasion Inion)	6.6 (12.7)	
Teeth	Second teeth beginning to form (many permanent teeth erupted in control).	
Vertebrae	Epiphyseal lines not visible (closed in control).	
Control	All epiphyseal lines are still open in the control puppy. Only lower ends of humerus and tibia are united.	
Tarsals and Carpals	Much more developed than at 5 months; carpals have little shape, tarsals are taking form. (In control all are well developed.)	
Penis	No bone in penis. (Well developed bone in penis of control.)	

Third series of x-rays was taken March 1, 1925, 39 weeks after operation.

	Hypophysectomized puppy H¹	*Control H²*
Length of femur	6.0 cm.	15.1 cm.
Length of tibia	6.0 cm.	16.1 cm.

In the hypophysectomized dog the proximal epiphysis of the femur is closed and the distal one is closing. No calcification in bone of penis. Permanent teeth are just erupting.

In control dog all epiphyses (of tibia and femur) are closed. Bone of penis is calcified. Permanent teeth have long been present (*cf.* table above at 18 weeks postoperative).

Weights

	Hypophysectomized H¹	*Control H²*
Time of operation	1.2 kilos	1.0 kilo
18 weeks after operation	1.7 kilos	7.4 kilos
39 weeks after operation	1.8 kilos	7.7 kilos
41 weeks after operation (at death)	1.4 kilos	

The chart (fig. 1) shows the weights and the lengths of tibia and femur during the life of these animals. The hypophysectomized animal gained 600 grams (mostly fat) during the 39 weeks after operation compared to a gain of 6700 grams in the control. During this period the operated animal showed only 4 cms. increase in the combined length of femur and tibia, while in the control the increase was 23 cms.

The hypophysectomized puppy always retained the long lanugo hair (figs. 2, 4); at least the hair was soft and downy at the skin, but many of the hairs became coarse distally. Curiously enough, the tail soon lost nearly all the hair and until death had the appearance of a rat's tail. This was also noticed in other operated puppies. The skin became a little thicker in the later months.

The testicles could never be seen or palpated, possibly owing to large pads of abdominal fat in the groins (fig. 3). The scrotum never developed.

Eight months after operation (when nearly nine months old) this puppy and the control were placed with a bitch in heat. The hypo-

physectomized puppy was entirely indifferent; the control behaved normally.

Although playful and fairly active the hypophysectomized animal always tired easily, much more so than a normal puppy of six weeks. It soon took on the general appearance of a senile animal (progeria)

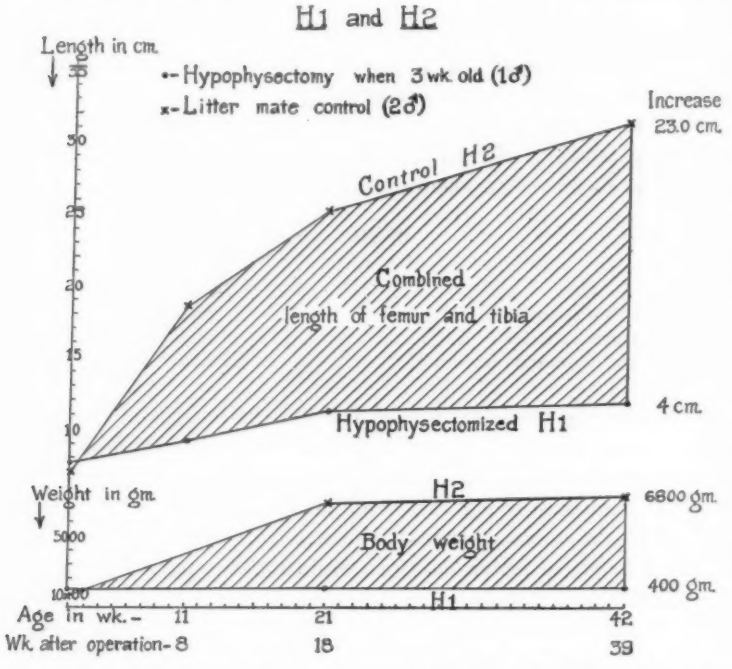

FIG. 1. CURVES SHOWING RELATIVE GROWTH OF BODY (LOWER CURVE) AND OF THE HIND LEG (UPPER CURVE) IN HYPOPHYSECTOMIZED PUPPY H^1 AND ITS LITTER-MATE CONTROL H^2

in spite of its puppy size. It was always snappy and cranky. Its bark was shrill and high pitched. It ran in a little trot but never jumped about in joyous play like a normal dog. In the later months of life it waddled as though tired and took little interest in anything except eating and resting. It knew its friends, wagging its tail in recognition; it barked in anger when strangers appeared. It died

March 26, 1925, of pneumonia, when eleven months old and forty-one weeks after operation. Autopsy showed very small organs, although all were well formed. Their size was roughly that belonging to a puppy of one month. No trace of thymus could be found. The left kidney and ureter were absent.

Unfortunately the weights and photographs of some of the organs have since been lost, but the following weights and measurements after formalin fixation were found: Adrenals, 1.5 gms.; right kidney (no left), 9.9 gms.; testicles, 0.85 gm.; lungs, 18.6 gms.; left femur, 6.35 gms.; left tibia, 5.0 gms.; length of large bowel, 9.0 cms.; of

FIG. 2. HYPOPHYSECTOMIZED PUPPY (RIGHT) H^1 AND LITTER-MATE CONTROL (LEFT) H^2, THREE MONTHS AFTER REMOVAL OF THE HYPOPHYSIS WHEN THREE WEEKS OLD

Its size has remained practically stationary. It still retains its baby hair. Contrast in vivacity between normal and hypophysectomized animal is most striking.

oesophagus, 12 cms.; total length of gastrointestinal tract, 87.0 gms. Serial sections were made of the base of the brain and no hypophyseal cells could be found.

Since permission could not be obtained to sacrifice the control at the time of death of the operated animal, sections of a three weeks' old puppy of approximately the same weight will be used for control. The histological appearance of the testes of the hypophysectomized puppy is that of immaturity. There are no spermatozoa, spermatocytes and only spermatogonia. There appears to be an almost total absence of the interstitial cells. In the adrenal which is smaller than

that of a six weeks' puppy no histological abnormalities can be detected. Nor do the histological appearances of the pancreas, liver, heart, kidney or spleen seem abnormal.

FIG. 3. H^1 AND CONTROL NINE MONTHS AFTER OPERATION
Photograph taken just before death of H^1

The chart (fig. 1) showing the growth and the photographs (figs. 3 and 4) illustrate the profound changes resulting from total hypophysectomy in the early weeks (third week) of canine life.

II. *H^{16} (total hypophysectomy) and H^{17} control.* H^{16} was a female fox terrier puppy; she was hypophysectomized September 1, 1927, when six weeks old. During the first six months after operation the puppy remained infantile, failed to grow, retained the puppy face and hair, failed to erupt permanent teeth (which occurred in the litter mate control H^{17} between the third and fifth months), and exhibited infantile genitalia. Six months after hypophysectomy (when seven and one-half months old) she showed an increase in weight of 1300 gms., whereas the control had increased 6700 gms. The growth in

FIG. 4. PHOTOGRAPH SHOWING IN MORE DETAIL THE PUPPY HAIR AND THE HAIRLESS TAIL OF THE HYPOPHYSECTOMIZED PUPPY H^1

the length of femur and tibia of H^{16} for the same period was 1.5 cms., while in H^{17} the increase was 9.0 cms. The increase in the length of the skull in H^{16} was 0.7 cm., and in H^{17}, 3.4 cms.

During the next four months pituitary replacement therapy (reported by Reichert) was given to the operated animal, her weight increased 800 gms., the same amount as in the control. The increase in length of femur and tibia of H^{16} during this treatment was 2.4 cms., and in the control (now nearly grown), 0.5 cm., and the resultant growth of skull of H^{16} was 0.7 cm., while in H^{17} it was 0.2 cm. Besides causing some growth until the epiphyses became closed in the treated

dog, the replacement therapy erupted the permanent teeth and changed the hair to a coarser and longer coat. Portions of the thyroid, adrenal and ovary were removed by operation in both H^{16} and H^{17} at the end of the period of replacement therapy. The histological picture of these organs in H^{16} is of interest, although the adrenals were not remarkable. The thyroid showed definite parenchymatous hyperplasia with several layers of cuboidal cells, the ovary (since the puppy was in a state of oestrus throughout the period of treatment) was larger and contained a large cystic corpus luteum, while the uterine endometrium was thicker and showed an excessive increase in number, size and tortuosity of the glands.

Subsequently the hypophysectomized animal lived to be $2\frac{1}{3}$ years old. There was no further growth; recession of the swollen genitalia occurred with the ending of replacement therapy. A growth and weight chart of H^{16} and H^{17} will not be reproduced here, since it is found in Chart VII, page 90 in Volume I of *Engelbach's Endocrinology*. The animal died of pneumonia and the control was sacrificed at the same time. Serial sections were made through the base of the brain; there was no trace of anterior or posterior lobe cells, but there was a small remnant of cells of the pars tuberalis.

The weights of the organs were as follows:

	H^{16}	H^{17}
Right thyroids	0.4 gm.	0.8 gm.
Adrenals	1.3 gms.	4.0 gms.
Pancreas	8.9 gms.	16.5 gms.
Spleen	10.5 gms.	21.6 gms.
Kidneys	29.5 gms.	64.5 gms.
Liver	150.5 gms.	369.0 gms.
Heart	54.0 gms.	73.5 gms.
Length of gastrointestinal tract	233 cms.	369 cms.
Sex, ovary and uterus, developed from previous replacement therapy	10.4 gms.	7.0 gms.

The organs, excepting those of sex, were all smaller than in the control, but histologically only the thyroid, ovaries and uterus differed noticeably from the normal. The thyroid was atrophic, showed small acini with flattened epithelium and much less colloid than the control. The breast tissue unfortunately was not saved for histological study.

The ovary, which had been affected for a short period a year and a half before death by replacement therapy, was now atrophied with only a few ova and follicles in the periphery surrounded by dense stroma—the control ovary had many ova and follicles and a fairly large cystic corpus luteum. The uterus was small and markedly fibrosed; there was a sparsity of glandular elements.

III. *H^{30} (total hypophysectomy) and H^{31} control.* Both were female litter mate puppies; H^{30} was totally hypophysectomized April 26, 1928, when four weeks old and died of pneumonia seven weeks later.

FIG. 5. LITTER-MATES H^{30} AND H^{31}. H^{30} HYPOPHYSECTOMIZED WHEN FOUR WEEKS OLD. FOUR WEEKS AFTER OPERATION

Although the period of survival was short, this experiment, together with that on H^{76} will demonstrate the comparative differences in growth, weights of organs and in the endocrine glands. Weekly measurements of skeletal growth were made from the roentgenograms; the table at the end of this section shows that growth was greatly inhibited two weeks postoperatively and was stopped by the fourth week. The growth of hair in a shaved area was almost twice as fast in the control as in the operated puppy. The weight of the control failed to increase at a normal rate because of the presence of intestinal worms. The photograph (fig. 5) shows a marked difference in size even in the

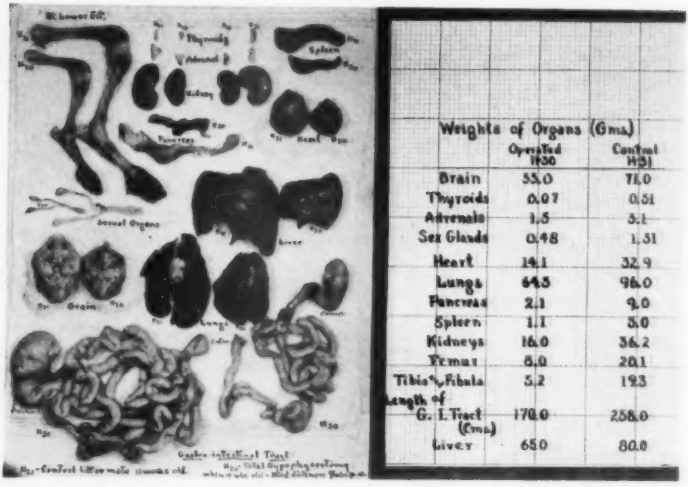

FIG. 6. PHOTOGRAPHS OF VARIOUS ORGANS (AND BONES OF HIND LEGS) OF H^{30} AND H^{31} 7 WEEKS AFTER HYPOPHYSECTOMY IN H^{30}

Weights are also appended

TABLE I

Measurements from x-rays at varying intervals after hypophysectomy

DATE AND WEEKS AFTER OPERATION	NO. H^{30}					NO. H^{31} (CONTROL)				
	Weight	Length of skull	Length of femur	Length of tibia	Length of femur and tibia	Weight	Length of skull	Length of femur	Length of tibia	Length of femur and tibia
	kilos.	*cm.*	*cm.*	*cm.*	*cm.*	*kilos.*	*cm.*	*cm.*	*cm.*	*cm.*
Operation, 4/26/28	1.19	8.3	5.3	4.7	9.0	1.98	9.7	6.6	5.5	12.1
5/ 4/28—1 week	1.42					2.5				
5/11/28—2 weeks	1.5	8.6	6.0	5.4	11.4	3.12	10.5	8.1	7.4	15.4
5/18/28—3 weeks	1.55					3.3				
5/24/28—4 weeks	1.5	9.0	6.4	5.8	12.2	3.18	11.0	9.1	8.1	17.2
5/31/28—5 weeks	1.56	Distemper				3.18	Distemper			
6/ 7/28—6 weeks	1.56					3.22				
6/15/28—7 weeks	1.24	9.0	6.6	6.2	12.8	3.12	11.3	10.1	9.3	19.4
Maximum gain since operation	0.37	0.7	1.3	1.5	3.8	1.24	1.6	3.5	3.8	7.2

fourth postoperative week. The photograph of the organs with their weights (fig. 6) shows the greatest difference in the thyroids, the sex glands, spleen, pancreas and adrenals. Histologically, too, marked differences can be seen in the thyroid and sex organs.

Serial sections through the base of the brain of H^{30} showed total absence of cells of the anterior and posterior lobes and the pars

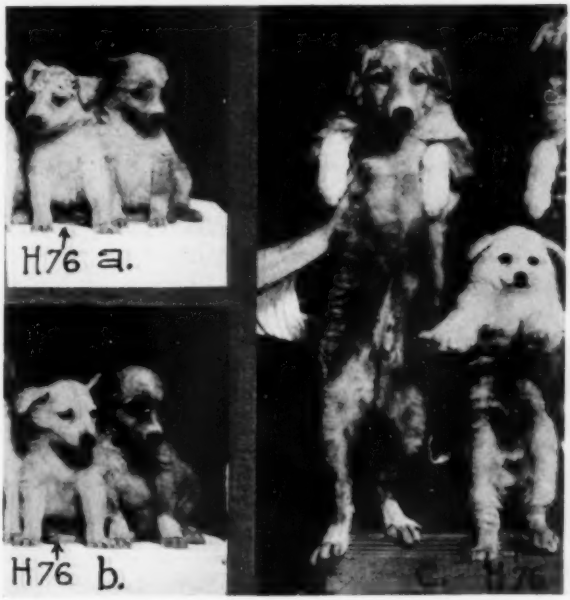

FIG. 7. PHOTOGRAPH OF H^{76} AND CONTROL H^{77}

(*a*) 2 days after total hypophysectomy of H^{76}; (*b*) 2 weeks after operation; (*c*) 10½ weeks after operation.

tuberalis. Histologically, the thyroid of H^{30} showed marked atrophy with diminution in the amount of colloid and in the size of the acini. The epithelium is flattened. The ovary of H^{30} showed atrophy, especially in the center, with a scant number of ova and follicles in the periphery in contrast to the sections of the control H^{31}. The uterus of H^{30} was smaller, with a thinning endometrium

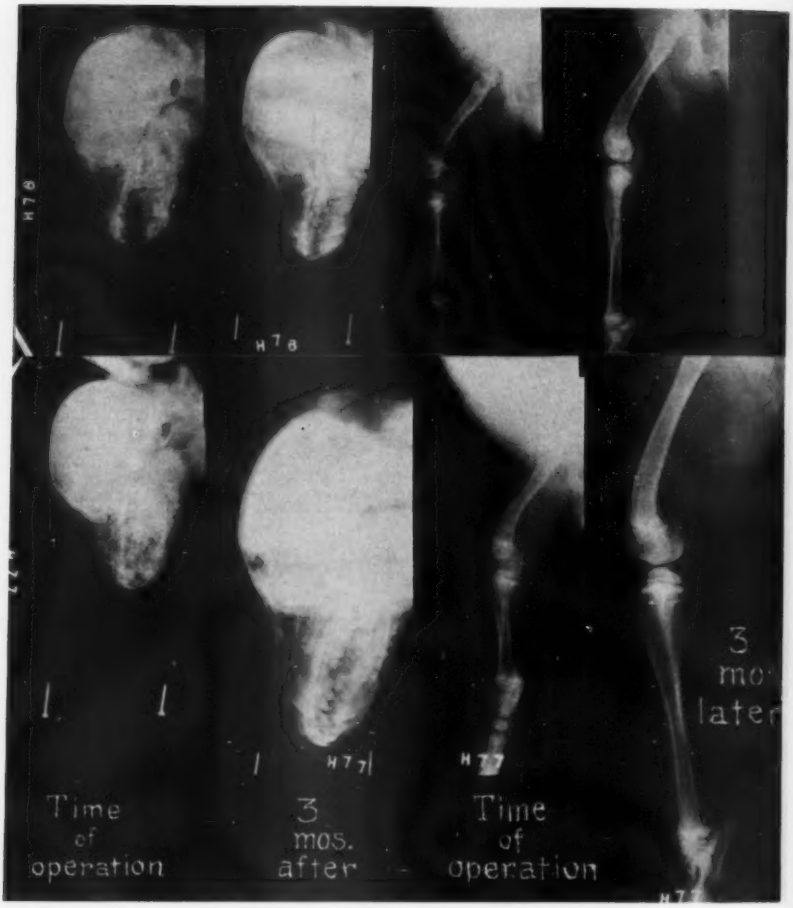

FIG. 8. PHOTOGRAPH OF ROENTGENOGRAMS OF HYPOPHYSECTOMIZED ANIMAL H^{76} (ABOVE) AND LITTER-MATE CONTROL H^{77}

The second and fourth figures in each row indicate the growth of the skull and of the bones of the hind leg at the period of three months following hypophysectomy of H^{76}.

and only a few glands. The adrenals, although of smaller size, showed no discernible histological change; the relation of cortex and medulla,

as well as the relative thickness of the three cortical zones, appeared to be the same. (See table I).

IV. *H^{76} (total hypophysectomy) and H^{77} (control).* These were litter mate female puppies of the collie type. When six weeks old H^{76} was totally hypophysectomized and the cautery applied to the base (July 25, 1929). The operated animal was sacrificed because of distemper thirteen weeks after operation, when nineteen weeks old.

FIG. 9. SECTION FROM BASE OF BRAIN OF H^{76} SHOWING CLUSTER OF PARS TUBERALIS CELLS

Here again the profound inhibition of growth is illustrated in the photograph (fig. 7) and in the roentgenograms of the skull and hind-limb (fig. 8) and in the accompanying table of growth curves.

Serial sections of the base of the brain of H^{76} revealed no cells of the anterior or posterior lobe, but there was a small cluster of pars tuberalis cells found (fig. 9).

As in H^{30}, the histological changes that were most marked were found in the thyroid and sex glands. The thyroid of H^{76} was quite different from that of the control; it showed marked atrophy of the

gland, smaller acini and a sparsity of colloid; the epithelial cells were smaller and flattened. The endometrium of the operated puppy was definitely more fibrotic and less cellular than the control. The pancreas seemed to have about the usual number of normal appearing islets and the adrenal, though smaller, showed the normal differences in thickness of the three zones; possibly there was a suggestion of a thinner cortex in relation to the medulla. No difference could be noted in the histological appearance of the spleen and liver. (See table II.)

TABLE II

Weights and measurements of bone from x-rays taken at intervals of about one week

DATE AND WEEKS AFTER OPERATION	NO. H^{76} (HYPOPHYSECTOMIZED)			NO. H^{77} (CONTROL)		
	Weight	Length of skull	Length of femur and tibia	Weight	Length of skull	Length of femur and tibia
	kilos	*cm.*	*cm.*	*kilos*	*cm.*	*cm.*
Operated 7/25/29, 6 weeks old	1.8	9.0	12.0	1.68	9.1	11.8
8/ 1/29— 1 week	1.9			2.0		
8/ 8/29— 2 weeks	2.1	9.8	13.5	2.3	10.4	15.4
8/13/29— 3 weeks	2.1			2.8		
8/22/29— 4 weeks	2.3			3.4		
8/29/29— 5 weeks	2.5			4.1		
9/ 6/29— 6 weeks	2.6			4.7		
9/12/29— 7 weeks	2.6			5.4		
9/19/29— 8 weeks	2.7			5.9		
9/26/29— 9 weeks	2.6			6.6		
10/ 4/29—10 weeks	2.6	9.8	15.0	7.1	13.6	24.5
10/10/29—11 weeks	2.5			7.0		
10/17/29—12 weeks	2.5			8.2		
10/24/29—13 weeks	2.3	9.9	15.0	8.2	14.5	26.5
Maximum gain since operation	0.9	0.9	3.0	6.52	5.4	14.7

V. *H^{99} (total hypophysectomy) and H^{100} (control).* A long-haired breed female puppy (H^{99}) was totally hypophysectomized and the cautery applied to the base when six weeks old (June 20, 1930). Eleven and a quarter months after hypophysectomy (May 5, 1931) H^{99} was used for another experiment showing the effect of daily injections of prolan and growth hormone in conjunction with Dr. H. M. Evans and his co-workers, and that experiment was reported in Section 12, in Volume II, *Memoirs of the University of California*, 1933 (3).

FIG. 10. (*a*) One week after total removal of hypophysis (H^{99}). H^{100} and H^{101} are litter-mate controls. (*b*) Photograph with litter-mate control H^{100} one month after operation, when ten weeks old. (*c*) and (*d*) H^{99} and H^{100} eleven and one-fourth months (45 weeks) after hypophysectomy in H^{99} when twelve and three-fourth months (51 weeks) old.

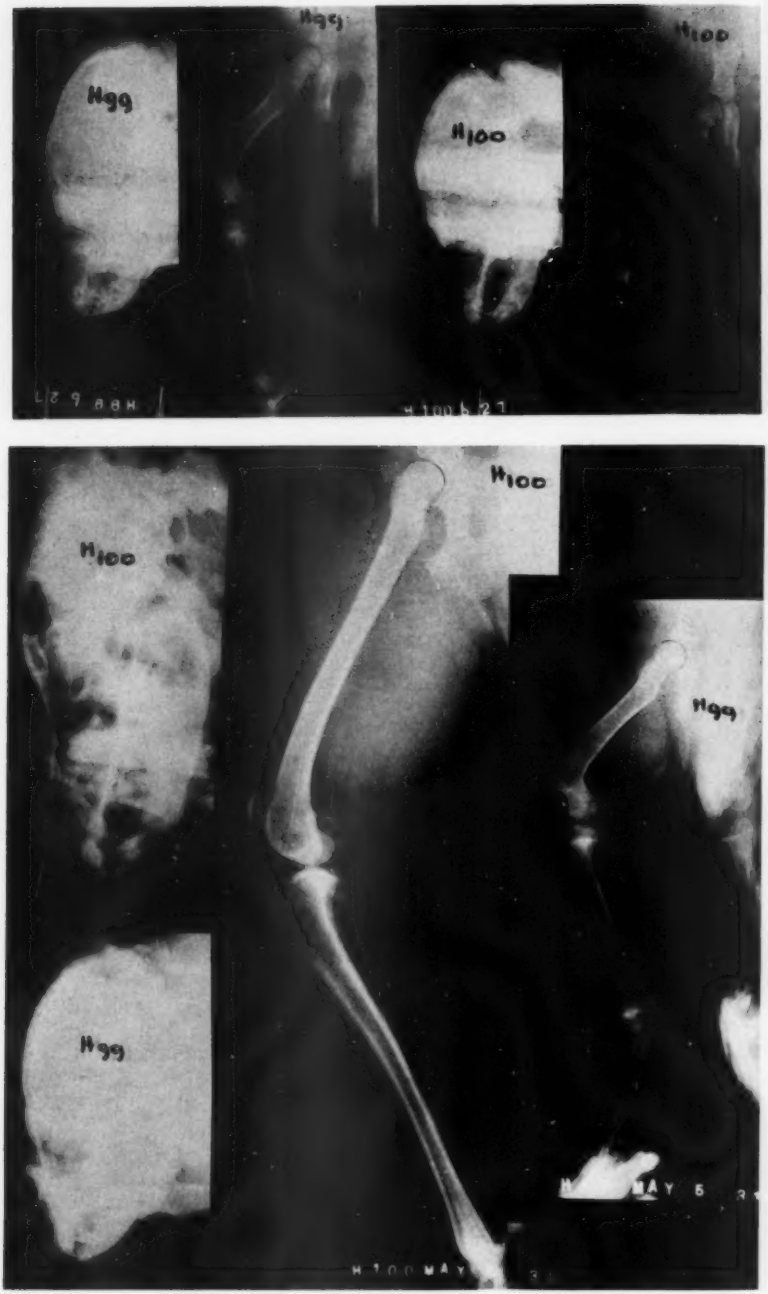

FIG. 11. ROENTGENOGRAMS SHOWING (ABOVE) SKULL, FEMUR AND TIBIA OF H^{99} AND ITS LITTER-MATE CONTROL H^{100} AT TIME OF HYPOPHYSECTOMY WHEN SIX WEEKS OLD

The lower roentgenograms show changes which have resulted in the same puppies nearly one year later.

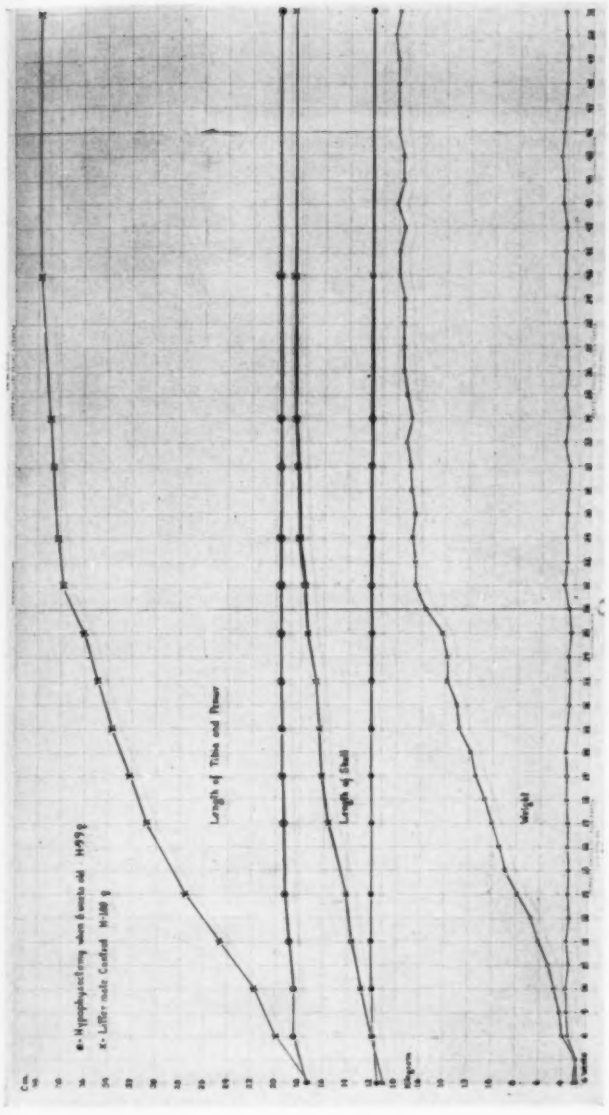

FIG. 12. COMPARATIVE GROWTH CURVES OF HYPOPHYSECTOMIZED ANIMAL H 99 AND ITS LITTER-MATE CONTROL H 100

Lower curves show the body weight, middle curves the length of skull and the upper curves the combined length of the tibia and femur.

Forty-five weeks after hypophysectomy H^{99} was still infantile, with puppy teeth and hair (the teeth in the control had changed at the age of three and one-half months); the epiphyseal lines were still open, though in the control they had closed at nine months.

Because of the subsequent experiment with the hormones the organ weights and the histological changes may possibly have been influenced by the therapy and are not used in this present report.

FIG. 13. MICROPHOTOGRAPH SHOWING PARS TUBERALIS REMAINS AT THE BASE OF THE BRAIN AT NECROPSY OF H^{99}

The photographs of these litter mate puppies at various intervals of time taken at the standard distance from the camera revealed the cessation of growth following hypophysectomy (fig. 10). The roentgenograms (fig. 11) show the failure of skeletal growth. The puppy teeth and the open epiphyses persisted nearly a year after operation. This cessation of growth is expressed graphically in the weight and growth chart (fig. 12).

Serial section of the base of the brain of the operated animal, H^{99}, showed a total absence of anterior and posterior lobe cells, but there was a small remnant of pars tuberalis cells at the base (fig. 13).

VI. *H^{92} (total hypophysectomy) and H^{93} (control).* A female litter mate collie breed puppy (H^{92}) was totally hypophysectomized and the cautery applied to the base when seven weeks old (April 28, 1930). It died of distemper forty-seven and one-half weeks after operation: the baby teeth, baby hair and high-pitched voice were still present;

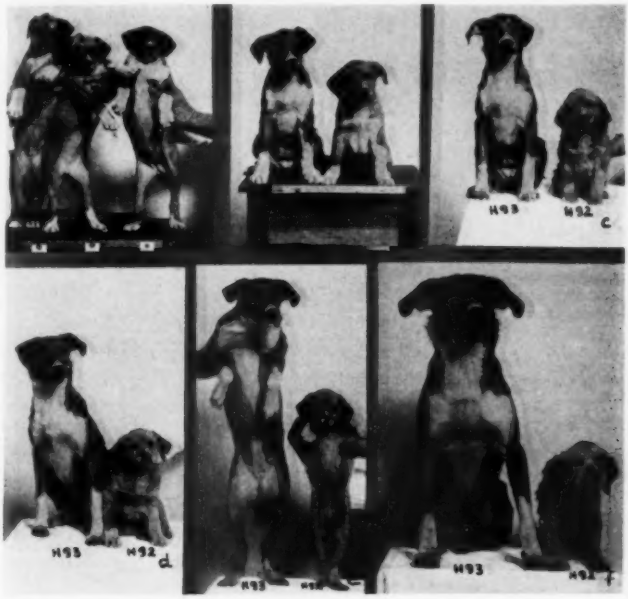

FIG. 14. SUCCESSIVE PHOTOGRAPHS (SAME DISTANCE FROM CAMERA) OF H^{92} AND ITS LITTER-MATE CONTROL H^{93}

(*a*) Two days after operation. (*b*) Six weeks after operation. (*c*) Thirteen weeks after operation. (*d*) Five and a half months after operation. (*e*) Five and a half months after operation. (*f*) Ten and a half months after operation.

the epiphyseal lines had remained open. The teeth in the litter mate control (H^{93}) changed at four and one-half months and the epiphyseal lines were closed at nine and one-half months.

The photographs of the animals (fig. 14) taken at the same distance from the camera, show the marked change with a striking senile appearance in the operated animal similar to that revealed in H^{1}

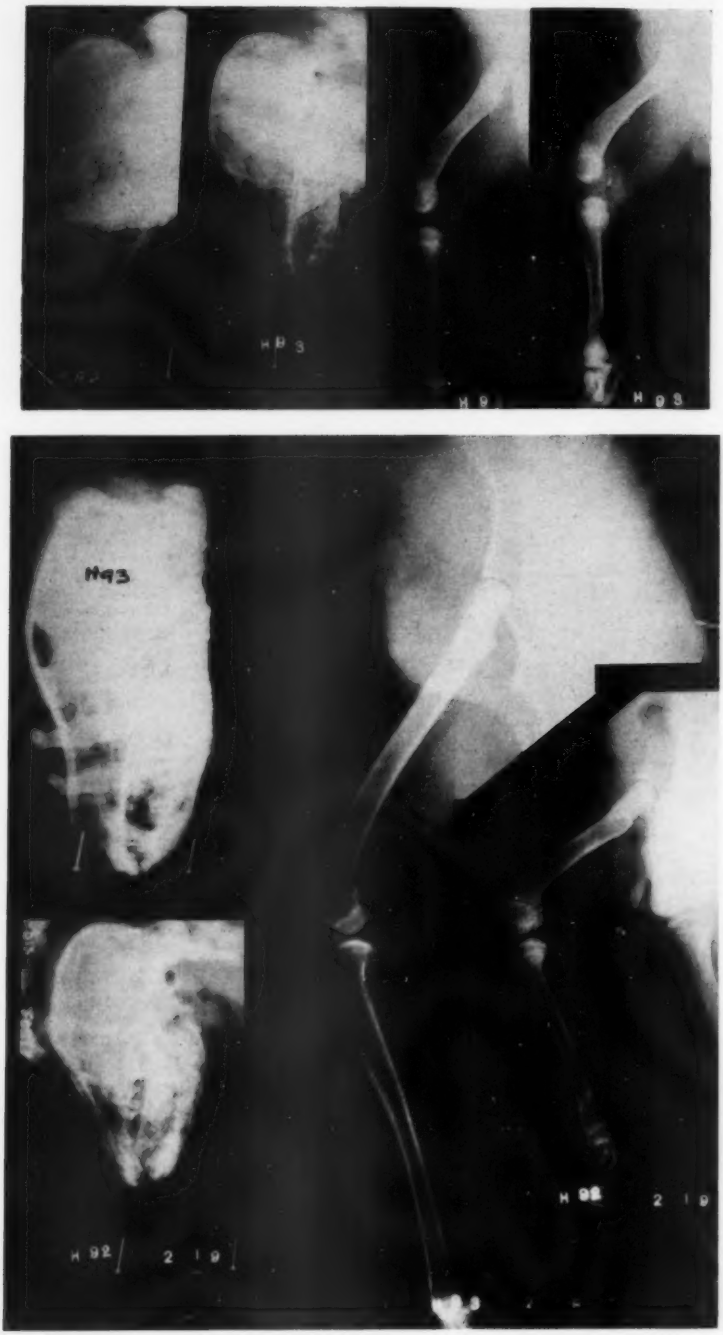

FIG. 15. ROENTGENOGRAMS (UPPER ROW) OF SKULLS, FEMURS AND TIBIAE OF H^{92} AND CONTROL H^{93} AT TIME OF HYPOPHYSECTOMY WHEN SEVEN WEEKS OLD

In lower row the roentgenograms indicate the changes one year later and show the epiphyseal lines closed in the control puppy (H^{93}), but still open in the hypophysectomized animal (H^{92}). The skull of H^{92} retained its puppy size and form.

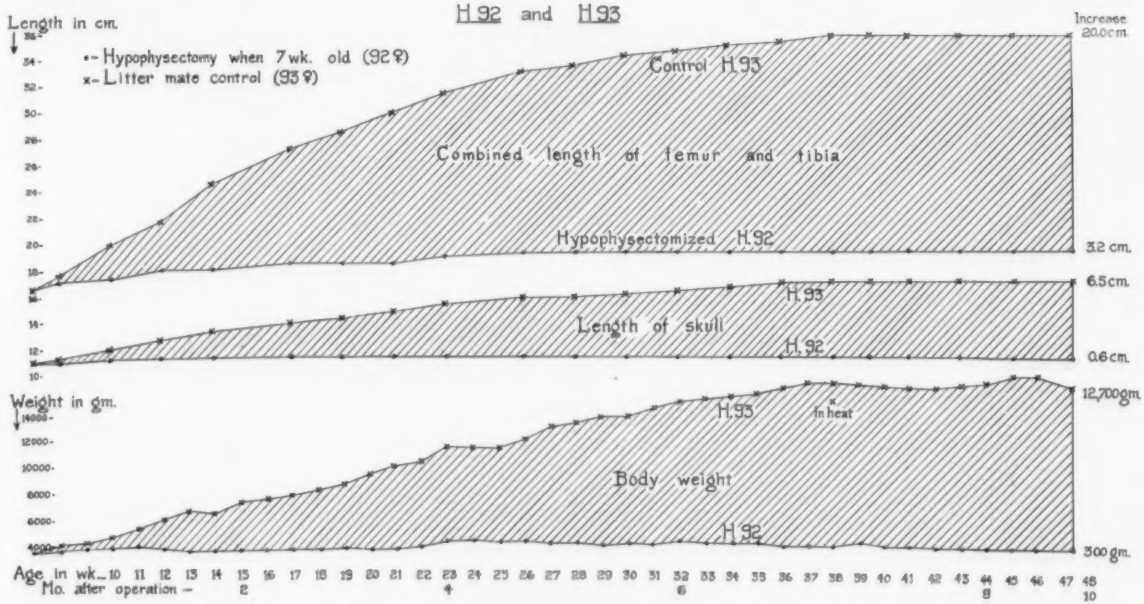
H.92 and H.93
Length in cm.
•-Hypophysectomy when 7 wk. old (92♀)
x-Litter mate control (93♀)
Increase
20.0 cm.
Control H.93
Combined length of femur and tibia
Hypophysectomized H.92
3.2 cm.
6.5 cm.
H.93
Length of skull
H.92
0.6 cm.
Weight in gm.
12,700 gm.
H.93
x
in heat
Body weight
H.92
300 gm.
Age in wk. 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
Mo. after operation — 2 4 6 8 10

FIG. 16. CHART SHOWING ACTUAL AND RELATIVE INCREASE IN BODY WEIGHT, LENGTH OF SKULL AND LENGTH OF HIND LIMB OF HYPOPHYSECTOMIZED PUPPY H^{92} AND ITS LITTER-MATE CONTROL H^{93}

and H^{99}. She was playful, combative and learned to follow tortuous and intricate passageways almost as quickly as her litter mate. But as she grew older she became deliberate in her gait and tired easily.

The photographs of the roentgenograms (fig. 15) of the two animals, taken at the time of the hypophysectomy and ten and one-quarter months after operation, show the retention in the operated

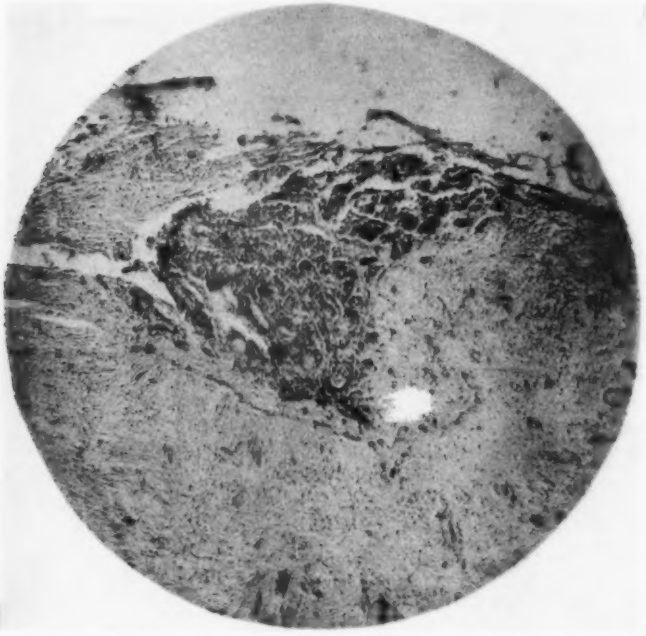

FIG. 17. MICROPHOTOGRAPH SHOWING SMALL REMNANT OF PARS TUBERALIS CELLS AT THE BASE OF THE BRAIN IN H^{92}

animal of the puppy shape of the skull, the first denture and the open epiphyseal lines. The curves of weight and growth (fig. 16) are similar to those of the others with total hypophysectomy.

Serial sections of the base of the brain in H^{92} showed a complete removal of the anterior and posterior lobes; a tiny remnant of pars tuberalis remained (fig. 17).

A study of the sections of the organs showed a small atrophied thyroid, with small acini and flattened cells. No difference could be seen in the parathyroids of the two animals. The ovary of the operated animal was very small, hard and almost entirely fibrotic; a few small ova could be seen in the periphery. The adrenal was small but showed no definite histological differences from the normal; the relative thickness of the three zones was essentially the same as the normal. The ovary contained no ripening follicles or corpora lutea. The breast tissue was small, atrophic and only occasional acini and ducts were visible.

Weight of organs

	H^{32}	H^{33}
Ovary, right (1.4 x 0.5 x 0.4 cm.)	0.225 gm. (both)	
Control (2.0 x 1.1 x 0.9 cm.)		0.92 gm.
Thyroids (1.7 x 0.6 x 0.4 cm.)	0.225 gm.	2.70 gm.
Adrenals (2)	0.705 gm.	3.6 gm.
Thymus	0.960 gm.	
Pancreas	10.850 gm.	35.4 gm.
Spleen	12.500 gm.	62.0 gm.
Kidneys (2)	43.4 gm.	127.5 gm.
Heart	49.8 gm.	158.5 gm.
Lungs (right lower lobe pneumonia)	103.0 gm.	Pneumonia
Liver and gall bladder	220.5 gm.	680.0 gm.
Gastrointestinal tract, weight	360.0 gm.	
Gastrointestinal tract, length	316 cm.	515 cm.
Brain (skull bone brittle)	70.2 gm.	98.5 gm.

VII and VIII. *H^{32} and H^{33} (subtotal hypophysectomy) and H^{32A} and H^{33A} (controls).* These were *subtotally* hypophysectomized puppies and had as their litter mate controls H^{32A} and H^{33A}.

Puppy H^{32} was a male, bred from an Esquimo bitch, which was hypophysectomized April 26, 1926, when five weeks old. The cautery was not applied to the base of the brain. At the time of the operation H^{32} weighed 1.8 kilos and the male litter mate control (H^{32A}) weighed 1.75 kilos.

Puppy H^{33} was a female of the same litter as the H^{32} puppy; it was hypophysectomized on the same day as H^{32}, when five weeks old. She weighed 1.85 kilos and her female litter mate control (H^{33A}) weighed 1.85 kilos. All of the puppies were kept in the country by an interested friend.

The first roentgenograms were taken August 14, 1926, fourteen weeks after operation and showed:

	Hypophysectomized		*Controls*	
	H^{32}, *male*	H^{33}, *female*	H^{32A}, *male*	H^{33A}, *female*
Length of femur	8.2 cm.	8.5 cm.	11.6 cm.	10.2 cm.
Length of tibia	8.3 cm.	8.1 cm.	11.2 cm.	10.4 cm.

The second roentgenograms were made October 10, 1927, seventy-six weeks after operation, when the puppies were eighty-one weeks old, and the following measurements were recorded:

	Hypophysectomized		*Controls*	
	H^{32}, *male*	H^{33}, *female*	H^{32A}, *male*	H^{33A}, *female*
Length of head	7.0 cm.			10.5 cm.
Length of femur	9.0 cm.	9.6 cm.		11.2 cm.
Length of tibia	8.9 cm.	9.7 cm.		10.9 cm.

Fourteen weeks after operation all the epiphyses were open in the hypophysectomized puppies; in the controls the proximal epiphyses of the femur were closed, and the remaining epiphyses of femur and tibia were open. At the age of eighty-one weeks (seventy-six weeks after operation) all epiphyses of both operated and control animals were united. The permanent denture had erupted in all animals at that time. Penile bone calcification occurred in the control when nineteen weeks old, but not in the operated puppy. It was calcified in the latter nineteen months after operation.

The recorded weights of the animals were as follows:

	Operated		*Controls*	
	H^{32}	H^{33}	H^{32A}	H^{33A}
At time of operation (5 weeks old)	1.8 kilos	1.85 kilos	1.75 kilos	1.85 kilos
19 weeks old (14 weeks p.o.)	2.3 kilos	3.3 kilos	4.0 kilos	2.0 kilos
1½ years old	4.21 kilos	4.3 kilos	5.0 kilos	4.3 kilos
At death, 2½ years old		6.0 kilos		

H^{32} died of pneumonia October 27, 1927, when one and one-half years old, and H^{33} died of pneumonia, August 22, 1928, when two and one-half years old.

Serial sections of the base of the brain in both operated animals, H^{32} and H^{33} showed small nubbins of anterior lobe, just visible to the naked eye.

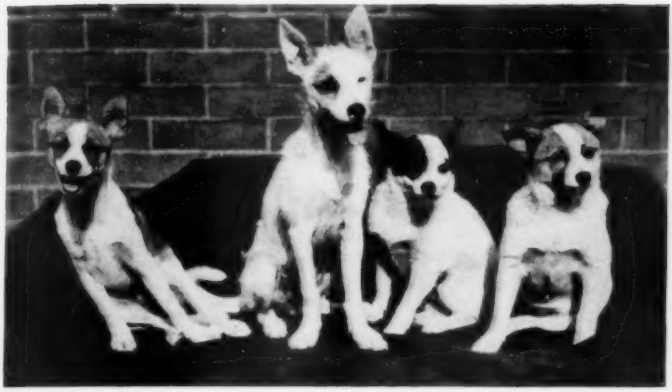

FIG. 18. H^{32} AND H^{33} AND CONTROL LITTER-MATES H^{32A} AND H^{33A}, NINETEEN MONTHS AFTER SUBTOTAL HYPOPHYSECTOMY ON H^{32} AND H^{33}

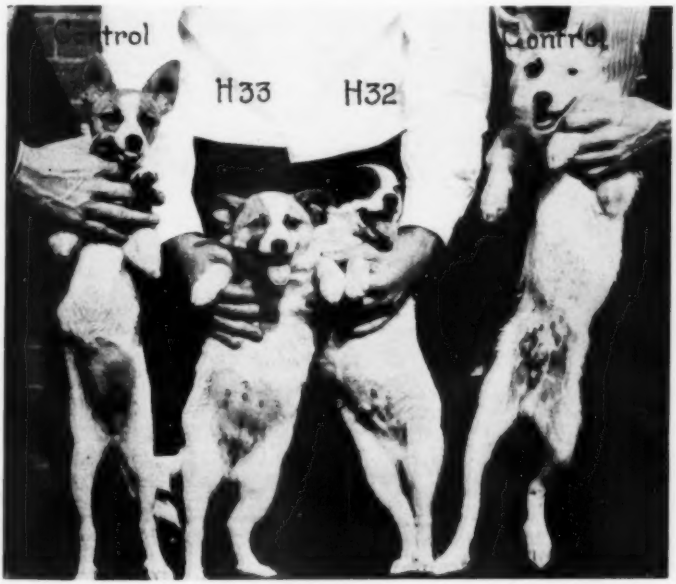

FIG. 19. VENTRAL VIEW OF SUBTOTALLY HYPOPHYSECTOMIZED (H^{32} AND H^{33}) AND LITTER-MATE CONTROL PUPPIES NINETEEN MONTHS AFTER OPERATION

The two operated puppies were very playful and alert, in marked contrast to the exhibition of premature senility exhibited in H[1].

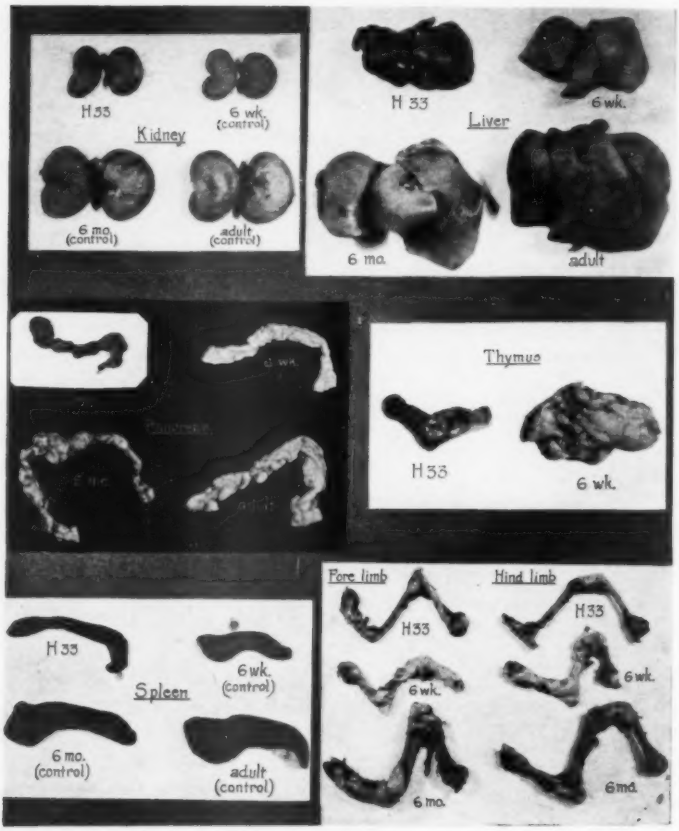

FIG. 20. PHOTOGRAPHS SHOWING COMPARATIVE SIZE OF VARIOUS ORGANS OF SUBTOTALLY HYPOPHYSECTOMIZED PUPPY H[33] TWO AND A HALF YEARS AFTER OPERATION, AND THOSE OF A NORMAL PUPPY AGED SIX WEEKS, A NORMAL PUPPY AGED SIX MONTHS, AND AN ADULT DOG

They became house pets and were bright, although they lacked the virility and extreme vivacity of the control puppies. At no time

before the last illness had either animal shown any signs of advancing senility and both would probably have lived much longer if intercurrent disease had not caused death. Their stature actually increased considerably, though falling far short of the controls, as seen in the photographs (figs. 18 and 19).

Unfortunately it was not possible to obtain the control animals to sacrifice at the time of the death of the operated puppies, but comparative studies by photography (fig. 20) and by weights of the organs

TABLE III

ORGANS	HYPOPHYSECTOMIZED		CONTROLS		ADULT
	H^{33}	H^{32}	At 6 weeks	At 6 months	
Body weight	3.0 kilos	1.5 kilos	1.4 kilos	3.2 kilos	5.7 kilos
Brain	52.5 gms.	46.5 gms.	49.0 gms.	67.5 gms.	63.5 gms.
Spleen	5.0 gms.	4.0 gms.	5.0 gms.	10.2 gms.	17.0 gms.
Liver	86.7 gms.	89.5 gms.	88.5 gms.	206.0 gms.	300.0 gms.
Kidneys (2)	14.8 gms.	16.5 gms.	15.0 gms.	50.5 gms.	46.0 gms.

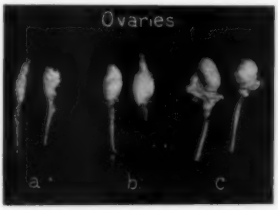

FIG. 21. PHOTOGRAPHS OF OVARIES OF (*a*) H^{33} TWO AND A HALF YEARS AFTER SUBTOTAL HYPOPHYSECTOMY; (*b*) PUPPY SIX WEEKS OLD; (*c*) PUPPY SIX TO EIGHT MONTHS OLD

were, therefore, made from (1) a normal six weeks old puppy, (2) a six months old puppy, and (3) an adult dog, all of fox terrier breed. It was felt that these animals essentially served the purpose of controls, since the ultimate size of the animals was almost the same as shown in table III.

Although not weighed, the photographs (figs. 21, 22, 23) of the ovaries, thyroids and adrenals of H^{33} show marked atrophy of the ovaries and thyroid when compared with a normal six weeks puppy, but the adrenals were about equal in size to that of a six weeks ani-

mal. The ovaries were small, hard and fibrous, but with quite a few immature ova in the periphery. The thyroids showed a marked atrophy with smaller acini and a diminished amount of colloid. The adrenals showed on definite histological differences from the normal.

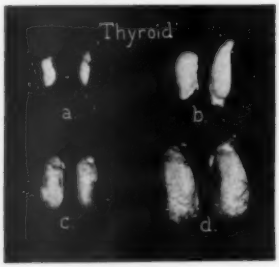

FIG. 22. PHOTOGRAPHS OF THYROID GLANDS OF (*a*) H^{33} TWO AND A HALF YEARS AFTER SUBTOTAL HYPOPHYSECTOMY; (*b*) PUPPY SIX WEEKS OLD; (*c*) PUPPY SIX TO EIGHT MONTHS OLD; (*d*) ADULT DOG OF ABOUT SAME SIZE AS CONTROL ANIMAL

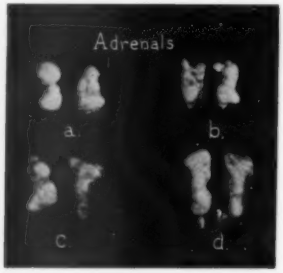

FIG. 23. PHOTOGRAPHS OF ADRENAL GLANDS OF (*a*) H^{33}, TWO AND A HALF YEARS AFTER SUBTOTAL HYPOPHYSECTOMY; (*b*) PUPPY SIX WEEKS OLD; (*c*) PUPPY SIX TO EIGHT MONTHS OLD; (*d*) ADULT DOG OF ABOUT SAME SIZE AS CONTROL ANIMAL

HISTOLOGICAL STUDIES

Under the microscope it is not possible to detect any abnormality in the heart, lungs, liver, spleen, pancreas, kidney or thymus. Histological deviations from the normal are found only in certain glands of internal secretion, *i.e.*, the thyroid and sex glands. The adrenals certainly are grossly changed as much as the thyroid but we can detect

no histological difference from the normal. No positive change could be seen in the parathyroids.

The ovaries and testes show all signs of immaturity. In the female subtotal hypophysectomies there are graafian follicles in varying degrees of development, but the number of such functioning follicles is tremendously reduced. However, from a microscopic inspection of the ovary alone one could predict that the hypophysectomy had not been total. None of the developing follicles were present in the totally hypophysectomized animals. It was difficult to find any glandular tissue in the breast.

In order to reach a purely objective and unbiased view concerning any possible histological changes in the adrenals and thyroids a test was made as follows: Six unlabeled sections of thyroid—(1) from total hypophysectomy, (2) and (3) from subtotal hypophysectomy, (4) from a normal dog of six weeks, (5) from a normal of six months, (6) from a normal adult dog—were given to Drs. Arnold R. Rich, Henry M. Thomas, Jr., and William F. Rienhoff, Jr. (all specialists on this gland), and each was able to pick out the total and subtotal specimens and in addition could tell which was from the subtotal hypophysectomy. Similarly sections of adrenals—(1) and (2) from total, and (3) subtotal, and (4), (5) and (6) from normals, six weeks, six months and adult respectively—were given to Drs. George A. Harrop and Arnold R. Rich, who have been especially interested in this gland, and neither was able to see any difference between the normal and hypophysectomized specimens.

SUMMARY AND CONCLUSIONS

The longest life span in our series of total hypophysectomies was two years and seven months, another two years and five months. The longest period of survival of any hypophysectomized dog in the literature was three years (Brown). Distemper and pneumonia were the usual causes of death. That they were more susceptible to the contraction and less resistant to the effects of disease than normal dogs is, we think, quite definite.

Dogs without a hypophysis definitely show signs of senility much sooner; indeed they very closely suggest the well known human state of progeria. Although playful, they are much less active and far less rough in their play. They appear to be bright, and they learn

and make good household pets. However, their mentality is unquestionably far lower than that of normal dogs.

These hypophysectomized puppies, besides remaining infantile in growth, retain their soft puppy hair, their shrill puppy bark, their first denture, their infantile external sexual apparatus, their playful investigative, combative and destructive puppy attitude. Growth of hair on the shaved head is delayed fully twice as long as on the control.

The almost total cessation of skeletal growth soon after total hypophysectomy is the most astounding of all changes. It appears quite probable that, from some unknown cause, there is a very slight skeletal growth for two to three weeks after the removal of the gland; after this the growth ceases entirely. This is in contrast to the immediate cessation of growth that has been shown to occur in rats (Smith). The younger the age of the animal at the time of hypophysectomy, the more striking the skeletal deficiency, probably because the relative growth is so much greater in the early weeks of life. Our case 1 (H^{1}) is probably the youngest successful hypophysectomy. It exhibits a much more striking "midget" than H^{92} which was operated on when seven weeks old, or Aschner's beautiful pioneer result (his figure 85) from the eighth week.

The closure of the epiphyseal lines was delayed to nineteen months in the subtotal hypophysectomized animals H^{32} and H^{33}, whereas in the other animals they remained open for over a year without any evidence of closure; in the controls the epiphyseal lines closed and growth ceased between the seventh and ninth months. When the control had reached adult life the epiphyses of the hypophysectomized dogs could be closed in from two to four months by anterior lobe replacement therapy as reported elsewhere (Reichert). The penile bone never calcified in totally hypophysectomized puppies and calcification was greatly delayed (nineteen months) in the subtotally hypophysectomized puppy H^{32}. Penile calcification in the normal dog begins at the fourth to fifth month.

Along with the cessation of skeletal development is a similar cessation of growth, perhaps in some glands an actual recession in size, of all organs of the body. In the table of organ weights of H^{30} and H^{31}, and H^{76} and H^{77} the brain, lungs and liver show relatively less difference in weight than other organs, probably because in the early

weeks of life they already have attained a proportionately larger size; the heart, spleen, kidneys, long bones and length of gastrointestinal tract show a greater disproportion, and the endocrine group of glands, *i.e.*, the thyroids, adrenals, sex glands and pancreas, show the greatest disproportion in weight and size.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Snow on Cholera, being a Reprint of Two Papers by JOHN SNOW, M.D., together with a Biographical Memoir by B. W. RICHARDSON, M.D., and an Introduction by WADE HAMPTON FROST, M.D. (*New York, the Commonwealth Fund, 1936.*)

The magnificent development of bacteriological methods has rendered them the most important tool for epidemiological research. This fact is so impressive that it often makes us forget the results which were achieved before the bacteriological era. Yet the work of John Snow is a classical example of the correct solution of an epidemiological problem by observation, interpretation of statistical data and clear reasoning. Studying the propagation of cholera, John Snow, then a London practitioner reputed for his skill in the application of anesthetics, was impressed by the rôle played by contaminated drinking water. He published his results in 1849 in a pamphlet "On the Mode of Communication of Cholera" of which an enlarged edition appeared in 1855. Before the discoveries of Pasteur and Koch he visualized an organic and specific virus entering by the digestive tract as the chief cause of the disease. In an address "On Continuous Molecular Changes, more particularly in their Relation to Epidemic Diseases" (1853) he developed his views into a general theory of contagion.

These two treatises together with Snow's biography written by his own friend, Richardson, are reprinted in the present volume. Credit for assistance in the arrangement of this interesting material must be given to Dr. Frost who, moreover, in an introduction gives a clear outline of Snow's place and significance in the history of epidemiology. By adopting the print and binding of the period, the book keeps in the spirit of the early Victorian era.

O. T.

An Introduction to the Social Studies. By JOSEPH K. HART. 203 pp. $2.00. (*The Macmillan Company, New York, 1937.*)

This elementary textbook for professional and preparatory groups is written with the idea of opening to view the various elements making up our present-day society, and of stimulating students toward sound social leadership. It is intended to be "a friendly guide along certain rather well-marked trails, into that real and actual world of social living and social problems."

In a chapter on the social professions, the author criticizes the attitude that the professional fields belong exclusively to the professions, and discusses briefly the relation of medicine to society. He mentions some of the controversial points

in this relationship as a problem to be considered by beginners in the field of social studies, and commends as an example of self-analysis the recent report of the American Foundation.

The book is clearly written, and although it touches on many large subjects, it gives a comprehensive view of the field of social studies which should serve well its purpose as an introduction, particularly in nursing and in premedical education.

G. C. R.

National Health Series. Funk and Wagnalls Company, New York, 1937. 35 cents each, or three for $1.00.

Cancer. By Francis Carter Wood. 102 pp.
Diabetes. By James Ralph Scott. 132 pp.
Food for Health's Sake. By Lucy H. Gillett. 74 pp.
Hear Better. By Hugh Grant Rowell. 83 pp.
Love and Marriage. By Thomas Walton Galloway. 102 pp.
Tuberculosis. By H. E. Kleinschmidt. 77 pp.
Why the Teeth? By Leroy M. S. Miner. 71 pp.
Your Mind and You. By George K. Pratt. 126 pp.
Staying Young beyond your Years. By Howard W. Haggard. 89 pp.
The Human Body. By Thurman B. Rice. 88 pp.

These ten small volumes serve well the aim to provide to laymen helpful and authoritative information about personal and community health.

Like the other ten volumes of this National Health Series, they may be recommended to those who seek light on the subjects with which they deal.

C. R. A.

The Business Side of Medical Practice. By THEODORE WIPRUD. 177 pp. Illus. $2.50. (*W. B. Saunders Company, Philadelphia, 1937.*)

The writer of this valuable little volume is not a physician. His early training had been in the business world, but for the last sixteen years he has had an intimate experience with physicians in city and rural practice, as Executive Secretary of the Medical Society of Milwaukee County, and Lecturer in Medical Economics at the Marquette University School of Medicine. He has been impressed with the lack of efficiency of the average physician in managing his office, keeping his case records and business accounts, as well as making judicious investments. Chapters are devoted to these subjects.

There are also sections on the behavior of the physician in court, the preparation of scientific papers and the correction of proofs, public speaking, the relation of the doctor with the press, methods of conducting a meeting, and the building up of a practice.

Physicians already established, as well as those just entering on their professional duties, will find very practical advice in this concise little treatise.

T. B. F.

BOOKS RECEIVED FOR REVIEW

Crippled Children: Their Treatment and Orthopedic Nursing. By EARL D. MCBRIDE and WINIFRED R. SINK. 2nd ed. 379 pp. Illus. $3.50. C. V. Mosby Company, St. Louis, 1937.

The Cerebrospinal Fluid. By H. HOUSTON MERRITT and FRANK FREMONT-SMITH. 333 pp. Illus. $5.00. W. B. Saunders Company, Philadelphia, 1937.

Biological and Clinical Chemistry. By MATTHEW STEEL. 770 pp. Illus. $8.00. Lea & Febiger, Philadelphia, 1937.

Methods of Treatment. By LOGAN CLENDENING. 6th ed. 879 pp. Illus. $10.00. C. V. Mosby Company, St. Louis, 1937.

The Management of Fractures, Dislocations, and Sprains. By JOHN ALBERT KEY and H. EARLE CONWELL. 2nd ed. 1246 pp. Illus. $12.50. C. V. Mosby Company, St. Louis, 1937.

The Practice of Medicine. By JONATHAN CAMPBELL MEAKINS. 1343 pp. Illus. $10.00. C. V. Mosby Company, St. Louis, 1936.

Genital Abnormalities, Hermaphroditism and Related Adrenal Diseases. By HUGH HAMPTON YOUNG. 649 pp. Illus. $10.00. Williams & Wilkins Company, Baltimore, 1937.

A Bibliography of the Works of Ambroise Paré. By JANET DOE. 266 pp. Illus. $5.00. University of Chicago Press, Chicago, 1937.

The Diagnosis and Treatment of Sexual Disorders in the Male and Female. By MAX HUHNER. 490 pp. $5.00. F. A. Davis Company, Philadelphia, 1937.

A Textbook of Eye, Ear, Nose and Throat Nursing. By ABBY-HELEN DENISON. Completely revised by Lyyli Eklund. 2nd ed. 367 pp. Illus. $3.00. The Macmillan Company, New York, 1937.

National Organization for Public Health Nursing. Board Members' Manual. 2nd ed. 173 pp. $1.50. The Macmillan Company, New York, 1937.

A Criticism of Nursing Education. By HAROLD BALME. 73 pp. Paper, 75 cents. Oxford University Press. London: Humphrey Milford, 1937.

Personnel Policies in Public Health Nursing. By MARIAN G. RANDALL. 170 pp. $2.00. The Macmillan Company, New York, 1937.

Surgical Pathology of the Diseases of the Neck. By ARTHUR E. HERTZLER. 237 pp. Illus. J. B. Lippincott Co., Philadelphia, 1937. (Hertzler's Monographs on Surgical Pathology).

Lane Medical Lectures: The Mechanism of Heat Loss and Temperature Regulation. By EUGENE F. DU BOIS. (Stanford University Publications. University Series. Medical Sciences, vol. III, No. 4). 95 pp. Illus. Paper, $1.50. Stanford University Press, Stanford University, California, 1937.

Short Years. The Life and Letters of John Bruce MacCallum, M.D., 1876–1906. By ARCHIBALD MALLOCH. 343 pp. Illus. $3.50. Normandie House, Chicago, 1938.

Die Diffusionsanalyse am Blutplasmagel. By RUDOLF BUCHER. 123 pp. Illus. Paper, 30 Swiss Fr. Benno Schwabe & Co., Basel, 1937.